S. HRG. 103–350

NOMINATION

HEARING

OF THE

COMMITTEE ON LABOR AND HUMAN RESOURCES UNITED STATES SENATE

ONE HUNDRED THIRD CONGRESS

FIRST SESSION

ON

HAROLD VARMUS, OF CALIFORNIA, TO BE DIRECTOR OF THE NATIONAL INSTITUTES OF HEALTH

NOVEMBER 3, 1993

Printed for the use of the Committee on Labor and Human Resources

U.S. GOVERNMENT PRINTING OFFICE

75–294 cc WASHINGTON : 1994

For sale by the U.S. Government Printing Office
Superintendent of Documents, Congressional Sales Office, Washington, DC 20402
ISBN 0-16-045860-9

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NOMINATION

WEDNESDAY, NOVEMBER 3, 1993

U.S. SENATE,
COMMITTEE ON LABOR AND HUMAN RESOURCES,
Washington, DC.

The committee met, pursuant to notice, at 10:05 a.m., in room SD–430, Dirksen Senate Office Building, Senator Edward M. Kennedy (chairman of the committee) presiding.

Present: Senators Kennedy, Simon, Mikulski, Wellstone, Kassebaum, and Coats.

OPENING STATEMENT OF SENATOR KENNEDY

The CHAIRMAN. The committee will come to order for the nomination hearing of Dr. Harold Varmus to be Director of the National Institutes of Health. I will just make a very brief opening comment and then recognize my colleagues.

The National Institutes of Health is internationally renowned as a symbol of America's preeminence of leadership in biomedical and behavioral research and the ongoing worldwide battle against disease in this country and many other nations.

The Director of the NIH administers an $11 billion comprehensive research program to improve the health of Americans through basic and applied biomedical and behavioral scientific research. The NIH consists of 18 research institutes, each with its own research focus.

Harold Varmus is an outstanding choice to lead the NIH. He has served with great distinction at the University of California at San Francisco, where he is a professor of microbiology, biochemistry and biophysics, and an American Cancer Society professor of molecular virology.

He came to the University of California at San Francisco in 1970 as a postdoctoral fellow and was appointed to the faculty that same year and became a full professor in 1979. He was a clinical associate at the National Institutes of Arthritis and Metabolic Disease from 1968 to 1970.

In 1989, Dr. Varmus shared the Nobel Prize for Physiology and Medicine as a result of his studies showing that cancer genes can arise from normal genes. He is the author of four books and nearly 300 scientific papers. He has served on a long list of boards dealing with important biomedical policy issues. He is a member of the Institute of Medicine and the National Academy of Science, the American Academy of Arts and Sciences.

Dr. Varmus is widely recognized for his ability to manage and to lead. His training as both a physician and a research scientist, his

scientific accomplishments, and his leadership experience make him an outstanding choice for the position of NIH Director. Throughout his brilliant career, he has demonstrated extraordinary commitment to scientific excellence. He has the vision and skill to lead the Nation's biomedical research into the 21st century, and we look forward to working closely with him. I look forward to recognizing him shortly, and will ask him to introduce his wife and members of his family at that time.

Senator Kassebaum.

OPENING STATEMENT OF SENATOR KASSEBAUM

Senator KASSEBAUM. Mr. Chairman, I would just like to say welcome to Dr. Varmus.

I think the National Institutes of Health is one of our most important institutions. It leads the way in biomedical research. Its focus is vital to this Nation, and is one of our real guiding lights.

I cannot think of anyone more distinguished to head the NIH at this time than Dr. Varmus who has made his mark in the field of biomedical research. Dr. Varmus will also be the first Nobel Laureate to head the National Institutes of Health. But more importantly, as I visited with him and examined his background I became impressed with his wonderful blend of scientific inquiry and his probing. He also has great compassion and enthusiasm for the work before him. Perhaps this comes from his undergraduate degree in English and his graduate degree in, I believe, Elizabethan poetry.

Dr. VARMUS. Seventeenth century, Senator.

Senator KASSEBAUM. All right. So his experience which perhaps will help him keep a perspective on the very important issues that he will be dealing with as head of the National Institutes of Health. Welcome.

Thank you, Mr. Chairman.

The CHAIRMAN. I might just say parenthetically that this was the area of interest of Neil Rudenstein. Neil was a Rhodes Scholar, is currently president of Harvard, and it is very interesting to hear him talk about those issues as well as many others.

Senator Simon.

OPENING STATEMENT OF SENATOR SIMON

Senator SIMON. I know of no Senators with that kind of background, I might say. [Laughter.]

Senator WELLSTONE. I actually have it, but I am modest, and I do not let many people know about it. [Laughter.]

Senator SIMON. But we do welcome you. Let me just mention one of the areas of difficulty that you face—and we create this in part because we see a special need, and we start championing that need. One of the things that you have to do is you have to say to Paul Simon or Paul Wellstone or Ted Kennedy or Nancy Kassebaum or Dan Coats, "This is important, but here is an area that does not have as much public appeal, and we also need to balance this." So it is important that you give us that sense of balance. Sometimes that is not easy when you are dealing with members of the Senate.

We welcome you. Your background certainly is an illustrious one.

The CHAIRMAN. Are there any further comments?

Senator KASSEBAUM. Mr. Chairman, I have statements of Senators Hatch, Thurmond, and Durenberger that they would like to have included in the record.

The CHAIRMAN. They will be so included.

[The prepared statements of Senators Hatch, Thurmond, and Durenberger follow:]

PREPARED STATEMENT OF SENATOR HATCH

Mr. Chairman, I have carefully reviewed the credentials of Dr. Varmus. He is eminently qualified for this position, so qualified, in fact, that each member of this committee should give Dr. Varmus a personal thanks for agreeing to accept the President's nomination.

Running an agency the size of NIH is not easy. I guess some would say that it pretty much runs itself, and that has been one of the problems out there.

Although Dr. Varmus has not had experience in managing an organization equalling the size of NIH, few in America have. His broad range of scientific expertise, the prestige of finally having a Nobel laureate at the helm of NIH, and his reputation for being a "scientist's scientist" will more than make up for that shortcoming.

Noting that Dr. Varmus has been teaching in San Francisco for the past two decades, I suspect we see in this nomination the fine hand of another who is familiar to this committee—Dr. Phil Lee. And so, I must commend Dr. lee, as well, for this excellent choice and Secretary Shalala for urging the President to send forward Dr. Varmus' nomination. I know he will not disappoint us.

Dr. Varmus, I fully intend to support your nomination and to be helpful to you whenever I can. It is an honor for this committee to act on your nomination.

PREPARED STATEMENT OF SENATOR THURMOND

Mr. Chairman, it is a pleasure to be here this morning. I would like to join my colleagues in welcoming Dr. Harold Varmus who has been nominated to be Director of the National Institutes of Health (NIH).

Dr. Varmus is currently a professor at the University of California at San Francisco, in the Department of Microbiology and Immunology. He earned a Bachelor of Arts from Amherst College and a Master of Arts from Harvard University. Dr. Varmus then earned his medical degree from Columbia University. He spent an early part of his career as a Clinical Associate at the National Institute of Arthritis and Metabolic Diseases.

As you know, the NIH is part of the Department of Health and Human Services. It functions as the principal biomedical research agency of the Federal Government. Within the National Institutes of Health are of a number of institutes responsible for research relating to specific causes. among these are: the National Cancer institute, the National Heart, Lung, and Blood Institute, and the National Institute of Diabetes and Digestive and Kidney Diseases.

Theses institutes foster and support basic and clinical research into the causes, treatment and prevention of various related diseases.

I recently met with Dr. Varmus and I was impressed with his knowledge of clinical research. Dr. Varmus, I would again like to welcome you here today, and I look forward to your testimony.

PREPARED STATEMENT OF SENATOR DURENBERGER

Thank you Mr. Chairman. Dr. Varmus, as the appointed Director of the National Institutes of Health you are taking the helm of an organization with a nearly $11 billion budget. NIH is the centerpiece for biomedical research in this country. NIH's grants to researchers in academia and industry, and its own cadre of top-flight scientists, have greatly contributed to American leadership in basic research.

Because of this leadership, I don't believe the NIH should stand on the sidelines of the health care reform debate. I would go as far as to say it can't. The quality of medical care patients receive is irreversibly linked to the successes of our basic medical research.

The purpose of scientific research conducted at the NIH is to benefit patient care and human health. Biomedical research should never lose sight of that goal. But health care reform is also teaching us that human health demands much more than basic biomedical or biochemical science.

As the NIH director you will face the challenge of the current health care policy environment to determine innovative, effective and appropriate patient treatments. The challenge requires a visionary strategic plan—a recommendation that your predecessor, Dr. Healy, took up, but a recommendation that has never been followed-through at the NIH.

That strategic planning should include nurturing of the highly innovative and rapidly growing field of biomedical engineering research. Biomedical engineering has contributed to the development of new medical devices that have revolutionized patient care. I requested that a study on the status of biomedical engineering be conducted by the NIH because of the fundamental importance of this field in our basic science arsenal.

The NIH seems to shy away from research on treatment outcomes. Maybe part of a strategic plan should include development of these research capabilities? I believe that with impending health care reform, the NIH cannot afford to ignore this critical area. At the least, the NIH should try to coordinate its basic research with outcomes measurements conducted by other agencies in the Department of Health and Human Services.

In the complex organization of the NIH, you also will be faced with continuing problems of morale of senior government scientists, potential and real conflicts of interest between publicly funded research and private industry, appropriate participation in clinical trials and issues of fair allocation of NIH resources among many competing constituencies. And in recent years, there have been allegations of scientific and sexual misconduct and racial prejudice. These problems demand sensitivity and deft managerial skills. The NIH cannot risk the appearance of a damaged institution if it is to continue to be a leader in basic research.

Dr. Varmus, I am sure you are aware of the enormous responsibility that you will assume as the NIH director. Your role is even greater because of the times. I encourage you to define the NIH's role in health care reform, and not to lose sight of creativity in research. NIH has made the United States a leader in basic research. Let NIH be the building block for the higher quality health care we all seek.

The CHAIRMAN. Senator Wellstone.

OPENING STATEMENT OF SENATOR WELLSTONE

Senator WELLSTONE. Mr. Chairman, I can be brief and just say that I would echo the comments of both Senators, or all of you, and say that I had a chance to meet with Dr. Varmus, and above and beyond just a brilliant background, a brilliant resume, and someone whom I think will provide brilliant leadership, there is also his sensitivity to people and his openness, which I think is very, very important. So I am just delighted to have him here today.

The CHAIRMAN. Senator Mikulski.

OPENING STATEMENT OF SENATOR MIKULSKI

Senator MIKULSKI. Thank you very much.

Dr. Varmus, we want to welcome you here to the Capital. You certainly are being introduced by two of the most distinguished colleagues on Capitol Hill, our good friend Senator Boxer, and of course, Congresswoman Pelosi with her deep roots in both Baltimore and Maryland—and California.

Dr. Varmus, I have a unique role here. Not only do I enjoy being a member of this committee, but as the Senator from Maryland I am also the Senator of NIH and the Senator for NIH. And we will be particularly interested in working with you to make sure that NIH really reinvents itself for the 21st century. It has had a distinguished history in terms of its biomedical research, its groundbreaking work in behalf of humanity, and yet at the same time we know that here we are in 1994, that it has to bring about many changes both in science itself and also in attitudes, and also for new resources.

We look forward to working with you in the area of women's health, aging in America, behavioral and environmental research. We feel that NIH is a jewel in the crown of Federal laboratories in Maryland.

We are concerned, though, that NIH sometimes might be adrift. It has been surrounded by controversy in a variety of areas, and now is the time to bring it back to what I know dedicated people at NIH want it to be as well as the people of the United States—the top biomedical research organization in the United States of America and in the world.

I know you have won one Nobel Prize, but we are looking forward to giving you a prize for reinventing NIH and look forward to working with you.

The CHAIRMAN. Thank you very much.

The CHAIRMAN. Senator Boxer, we are delighted to welcome you here. We know how interested you are in research programs and

health-related issues, and we are delighted to hear from you this morning.

STATEMENT OF HON. BARBARA BOXER, A U.S. SENATOR FROM THE STATE OF CALIFORNIA

Senator Boxer. Thank you so much, Senator Kennedy, and it is a pleasure to be here with my colleague, Congresswoman Nancy Pelosi. We seem to be doing this quite regularly because we have such wonderful nominees from California, and we are certainly very proud of this one.

First, I would ask unanimous consent that Senator Feinstein's statement be included in your record.

The CHAIRMAN. It will be so included.

Senator Boxer. Thank you.

And I send her best wishes, Dr. Varmus, to you.

Mr. Chairman and members of the committee, it certainly is a great pleasure to officially introduce you to Dr. Harold Varmus, President Clinton's nominee for Director of the NIH.

From what I have heard of your comments, I think you are very enthusiastic, so I will be brief in this introduction. I would say that in the Washington Post on August 9th, a leading researcher at the NIH gave a wonderful reaction to Dr. Varmus' nomination by saying, "I am ecstatic." I think most of us agree with her assessment, and I trust you will as well.

For those in the medical field, Dr. Varmus needs no introduction. He is a Nobel Prize winner for his work on cancer-causing genes, a leading researcher of retroviruses, a challenge we really face today, a distinguished professor with the University of California at San Francisco and the American Cancer Society, and the author of four books and nearly 300 scholarly scientific articles.

In contrast to many of his predecessors, Dr. Varmus is a research scientist who brings a wealth of experience to this job. Early in his career, Dr. Varmus served as a clinical associate at the National Institute of Arthritis and Metabolic Diseases. Since then, much of his own research has been supported by NIH grants. Dr. Varmus therefore knows the value of the NIH as an institution and the importance of its mission.

As a leader in the wider biomedical research community, Dr. Varmus is chairman of the board of biology for the National Research Council, an advisor to the Congressional Caucus for Biomedical Research, a member of the Joint Steering Committee for Public Policy of Biomedical Societies, and co-chairman of the New Delegation for Biomedical Research.

Dr. Varmus is respected by his peers both for his intelligence and his commitment to furthering science as an academic discipline. His friends and colleagues certainly have expressed it well.

The same article from the Post which I quoted earlier contained a statement from Alan Friedman, who heads the New York Hall of Science: "Varmus could become an articulate and passionate spokesman for the sciences. Science has lacked such advocates and suffered for it."

His research partner, co-winner of the Nobel Prize, and close personal friend, Dr. Michael Bishop, summed it up in the New York Times: "I think it is an outstanding appointment These are hard

times for science, and it is going to be good for morale that someone from the community of active, practicing scientists has taken the job."

Mr. Chairman, Dr. Varmus is truly a remarkable man. He was a liberal arts major, as Senator Kassebaum has pointed out, and he earned his master's degree in English from Harvard, Mr. Chairman—we needed to tell you that in order for you to be enthusiastically for this candidate—and then he applied and was accepted into medical school.

He is a Renaissance man for our times and will make an excellent director of the NIH. We need him because we are faced with such serious health challenges today. We all know what they are. Senator Mikulski has mentioned some. They include AIDS, Alzheimer's, cancer, all of the things that puzzle us, and we need his leadership.

We are proud to introduce you to the committee, Dr. Varmus.

The CHAIRMAN. Thank you very much, Senator Boxer, for coming to speak with us and urging quick, favorable approval.

[The prepared statement of Senator Feinstein follows:]

PREPARED STATEMENT OF SENATOR FEINSTEIN

It gives me great pleasure to support Doctor Harold Varmus of the University of California at San Francisco as the President's nominee as Director of the National Institutes of Health.

Doctor Varmus is an outstanding nominee. His biomedical research has already proven invaluable to medicine, and his diverse experience will greatly benefit the National Institutes of Health.

Dr. Varmus is the recipient of numerous honors and awards, including a 1989 Nobel Prize for medicine and Physiology. His work with Doctor J. Michael Bishop confirmed the genetic origin of cancer cells, which has lead to new discoveries in detecting the disease and developing appropriate treatments.

Since 1984, Doctor Varmus has been an American Cancer Society Professor of Molecular Virology. He has written four books and testified before congressional committees numerous times as an expert panelist. Doctor Varmus has also researched retroviruses—including the virus that causes AIDS.

Dr. Varmus will take an innovative approach to overseeing the Nation's premier biomedical advancement. For these reasons, it is my honor to support Doctor Varmus' nomination as Director of the National Institutes of Health.

The CHAIRMAN. We now want to welcome a long-time friend of mine and a wonderful leader on many health-related issues and a strong supporter of research programs.

We are delighted to have you here, Congresswoman Pelosi.

STATEMENT OF HON. NANCY PELOSI, A REPRESENTATIVE IN CONGRESS FROM THE STATE OF CALIFORNIA

Ms. Pelosi. Thank you very much, Mr. Chairman and members of the committee. It is obviously a great pleasure for me to be here and offer my strong support for Dr. Varmus, joining my Senator, Senator Boxer—I like to say it as often as possible; it is music to my ears—and of course, joining with our other Senator, Senator Feinstein, with her message as well, for Dr. Varmus' confirmation as Director of the National Institutes of Health.

I am honored to be here to join in introducing him, and his family is here as well, and I know he will reserve the right to introduce them, but that is so much a part of his life that I believe it needs mention as well as the fact that he is a Renaissance man. All of us who know him in San Francisco know that to be true.

He lives in my congressional district in the city of San Francisco, where most recently we were fortunate to have him serve as professor of microbiology, biochemistry, biophysics, and molecular virology at the University of California Medical Center, San Francisco, UCSF.

Senator Mikulski, Dr. Varmus' accomplishments in medical and scientific fields are significant, as you indicated, and his contributions to health are exceptional. Based at UCSF for the past 23 years, Dr. Varmus is recognized the world over as an authority on retroviruses and the genetic basis of cancer, as your colleague Senator Boxer mentioned.

Pending the Senate's confirmation, Dr. Varmus will become the first NIH Director to have won the Nobel Prize, as was indicated here, an honor which was awarded him in 1989 for his research on cancer-causing genes. In addition, Dr. Varmus was an early force in AIDS research, serving in 1986 as chairman of the subcommittee of the International Committee on the Taxonomy of Viruses that gave the AIDS virus its name. Most recently, Dr. Varmus has focused on the biochemical properties of HIV.

I am here as the representative from San Francisco and as a strong supporter of the NIH and a strong advocate for Dr. Varmus, but I must also add that I come as a member of the Labor-HHS Subcommittee of the Appropriations Committee, where in addition to all of these attributes that we have been hearing about Dr. Varmus, I can testify that he is a very effective advocate for biomedical research and for his point of view. He has dedicated many years and brought much insight to our committee on these issues. You know the issues that he has worked on, and I would once again mentioned AIDS and breast cancer.

Mr. Chairman, a thorough discussion of the awards, honors, and achievements of Dr. Varmus would keep us here all day. His record, with which you are familiar, speaks for itself. I commend President Clinton for nominating such an outstanding candidate to direct the increasingly important functions of the NIH, which is located in Senator Mikulski's State of Maryland.

Dr. Varmus possesses an exceptional knowledge of the medical sciences, experience as an acclaimed medical researcher, and the dedication to improving the quality of life for all. I offer my strongest support for speedy confirmation of Dr. Varmus as Director of the National Institutes of Health and thank you for the opportunity to bring these thoughts to you today.

The CHAIRMAN. Thank you, Congresswoman Pelosi, for coming over and joining us to make this presentation. We are very grateful to you. It is nice to see you again.

Ms. Pelosi. Thank you very much, Mr. Chairman.

The CHAIRMAN. Dr. Varmus, you are off to a flying start. We look forward to hearing from you.

Would you care to introduce the members of your family?

STATEMENT OF DR. HAROLD VARMUS, DIRECTOR-DESIGNATE, NATIONAL INSTITUTES OF HEALTH

Dr. VARMUS. Yes, if I may. I would like to introduce my spouse, Constance Casey; and my younger son, Christopher Varmus, a stu-

dent at Georgetown Day School. I am pleased they were able to take some time from work and from school to be with me today.

The CHAIRMAN. Very good. We are delighted to have them.

Dr. VARMUS. Senator Kennedy, Senator Kassebaum, and other members of the committee, I am honored to appear before you today as President Clinton's nominee to head one of our country's greatest assets, the National Institutes of Health.

My preparation for this job, as you have heard, has been unusual. For most of my adult life, I have been an academic scientist, studying retroviruses and cancer genes, teaching graduate and medical students, and training postdoctoral fellows at the University of California, San Francisco, which we know informally as UCSF.

Given this background, I would like to explain why I want to take on the responsibilities of running an immense institution, why I believe I am prepared to do it, and what I hope to achieve.

I grew up in an atmosphere that encouraged public service in the health professions. My mother was a psychiatric social worker, active in community affairs in my home town, Freeport, NY. My father was a family doctor who also served as the Jones Beach State Park physician for about 30 years.

In this climate, it was natural that I would consider a career in medicine. But as an undergraduate at Amherst College, I developed a love of literature that I set aside only after a year of graduate studies.

My indecision about careers did not end there. I began Columbia Medical School, fascinated with the brain, intent on pursuing a career in psychiatry or neurology. A new interest in tropical medicine took me to a mission hospital in India. By the time of my residency, I was convinced that I wanted to practice internal medicine.

The NIH then pointed me in a new direction when I served as a Public Health Service officer at the NIH campus in Bethesda. My mentor, Ira Pastan—who, incidentally, is still at NIH—showed me how to use a simple model organism, the bacterium E. coli, to study a complex physiological phenomenon, hormone action. This experience converted me into an enthusiastic bench scientist, so I sought further research training and finally a job as a professor in a basic science department of the University of California San Francisco.

In this new setting, I used another kind of simple microbe, in this case a retrovirus, to study the genetic basis of cancer and the way genes behave in animal cells.

Although I left Bethesda in 1970, I did not leave the NIH. As a new faculty member, a large part of my salary was paid by an NIH Career Development Award, and for over 20 years, most of my laboratory's work, like the work of most laboratories in academic institutions, has been financed by grants from the NIH. I have been fortunate. With NIH funding, I have worked unimpeded by anything other than my own limitations. I have known the joys of discovery, I have nurtured brilliant students, and I have received public accolades for work that was largely an act of love.

The indebtedness I feel toward the NIH is one of the reasons I am sitting before you this morning.

In 1989, my colleague Mike Bishop and I shared the Nobel Prize in Physiology or Medicine for our discovery that viral cancer genes are derived from cellular genes. One unexpected consequence of this honor was a sudden and widespread interest in my views. As a result, I have spoken out or taken action on a wide variety of topics—the funding of young investigators, indirect cost reimbursements, the training of new scientists, and science education for the public, among others. I have been especially concerned about the need to explain why fundamental research in biology and chemistry is essential to our progress against cancer, AIDS, and many other diseases, and why fundamental research is essential to the biotechnology and pharmaceutical industries.

These new activities have helped to make me a candidate for the NIH directorship. But what qualities and what aspirations would I bring to the job?

As a working scientist, I will bring to discussions of science policy an intimate knowledge of how science is done and a firm commitment to scientific excellence.

As an investigator who has seen the pursuit of an obscure chicken virus create a new vision of human cancer, I will defend open-ended basic science against the calls for restricted applications of what we already know.

As a fair-minded citizen concerned with the role of science in our society, I will try to improve science education at all levels and promote the careers of women and minority scientists.

And finally, as a medically-trained custodian of Federal funds, I will encourage NIH investigators to extend their biological discoveries to clinical settings.

These are large challenges, especially in a time of fiscal constraint. But it is also a time of remarkable exuberance in biology, when our understanding of life forms is reaching heights that could not have been imagined 50 or even 20 years ago. We are learning the instructions written into our genes; the way our cells divide and our organs mature; and the precise damage to molecules that causes disease.

I welcome the stewardship of NIH, for the NIH remains the world's best hope for sustaining this progress and for realizing its dividends for human health.

Mr. Chairman, I thank you again for this opportunity to be here today, and I would be pleased to answer any questions that you and your colleagues might have.

The CHAIRMAN. Thank you very much.

We have been notified that we are expecting a series of votes at 11 o'clock which will interrupt this process. What we will try to do is to divide the time before that and submit some questions. If there are members who want to come back and work through that process, we will do the best we can. But we apologize. We are a day late getting started on your nomination, and we apologize in advance for that procedure.

We will try to do 6 minutes each, and I will ask staff to keep track of the time.

We were fortunate in Massachusetts—first, I enjoyed very much the opportunity to talk with you in my office just after the award of Nobel Prize to two researchers who were working in combina-

tion. I asked you at that time what kind of support the NIH was giving to those individuals who were directing research programs. At that time, you yourself mentioned the importance of that kind of funding. And I think it is enormously important to the American people to know that the kind of support they are giving to the NIH is resulting in this absolute excellence in terms of research.

Dr. Varmus, can you perhaps anecdotally comment about whether those kinds of programs which you mentioned are exceptional, or is it really a strong backbone to some of the breakthroughs that have been taking place over the past years?

Dr. VARMUS. As you know, Senator, NIH has been responsible for the training of the vast majority of scientists not only in this country, but a very large number abroad. The case of Philip Sharp, who is one of our Nobel Prize winners this year, is particularly illustrative. Dr. Sharp attended a small college in Kentucky where he studied chemistry and then was supported as a graduate student by NIH training funds. His research career has been underwritten over about 25 years by NIH grants of very modest size which have allowed him to pursue his interest in the basic mechanism by which genes are expressed. He started by studying a virus that one might think not to be terribly important medically, and by simply asking the probing questions about the peculiarities of the way that virus grows he made a discovery that has completely changed our conception of how genes are constructed in all our cells. It is this general ability of undirected NIH funding to support scientists with brilliance and ideas that has accounted for the dominance of American science today.

The CHAIRMAN. Do you have ideas or suggestions about how we can make scientific or technology advances developed at the NIH more available for patient care?

Dr. VARMUS. I think one of the things we are all seeing in just the last 5 years in medical research, in part as a consequence of the attention paid to the human gnome, is the development of tools to ask fundamental questions with human materials. This has produced a new field whose buzz word is "molecular medicine."

Molecular medicine is a field of biomedical research in which the revolution in molecular biology is now closely linked to our efforts to understand human disease at its most basic level.

What we need to reap the fruits of this new endeavor is to train people who can reach from the basic research arena to the bedside. We have done this in part through the medical scientist training program that is currently funding 800 students at a time to obtain both the M.D. and Ph.D. degrees. We are doing this through graduate programs at various institutions that instruct Ph.D. candidates in some of the clinical aspects of the kinds of basic problems they are working on.

At the NIH, of course, there is a very strong tradition of using the patients who come there for help as subjects for study that leads to the alleviation of their disease. And I hope that as we see the maturing of this field of molecular medicine that NIH, both intramurally and extramurally, will be playing a major role in bringing to the benefit of patients our advances in basic science.

The CHAIRMAN. Just personally, my son Teddy had osteosarcoma and was enrolled in an NIH program that involved only 22 chil-

dren, and about halfway through that, they certified it as a regime which was justified in terms of that support. And I am sure everyone on this panel has had his own experience.

My time is running out. I just want to mention a couple of areas. One is the issue of racial discrimination, to which I hope you will give some focus and attention. We are going to be dealing with mental health and substance abuse issues. As part of last year's reorganization, we created the Substance Abuse and Mental Health Service Administration to conduct service delivery under the Public Health Service. The research portion came to NIH, we are hopeful you will focus attention on the linkages between them. Also, the Office on AIDS Research; I understand that the search for a director of that office is underway, and we have spoken about that issue.

More recently, this committee has been very interested in the disability movement. Rehabilitation, with all of the possibilities that NIH research can have for positive impact on the functional capacity and ability of those persons with physical disabilities to live independently is something which I think is enormously important.

Another area of concern is the FIAU drug trial. The committee was obviously saddened to learn about the deaths of 5 of the 15 patient, and we would be interested in that review and what recommendations and suggestions you would have.

There are a number of other questions, but we will submit those.

Senator Kassebaum.

Senator KASSEBAUM. Thank you, Mr. Chairman.

Dr. Varmus, the Director of NIH in some ways has limited powers because each of the NIH institutes receives directly appropriated funds. However, as Director, you have a limited discretionary fund for emergent diseases, and a one percent funding transfer authority across institute lines.

How do you plan to strengthen the role of the NIH Director, and are there any plans that you or Dr. Shalala have to change or strengthen the role of the NIH Director?

Dr. VARMUS. Well, there are a number of things worth commenting on in response to your question, Senator Kassebaum. First of all, there are administrative things that could be done, and I have discussed them with Secretary Shalala and with Assistant Secretary Lee, that would give the NIH Director more authority to make appointments at NIH and to make appointments with salaries that are commensurate with the salaries that are available in academic institutions. If we can obtain such powers at NIH, that would give the NIH Director a good deal more authority, and of course, a certain amount of respect for having obtained those privileges from the Secretary. We are hopeful that those things will be possible in the near future.

The second issue relates to the way in which the Director coordinates research activities on the NIH campus. It has been my feeling for some time that we need to have more trans-institute activities, and those can be guided effectively by scientific leadership from the Director's office. And I hope in response to a previously issued plan or report by intramural scientists and to ongoing reviews of the intramural program that are occurring as we speak,

that I will be able to bring some of the research activities that go on in different institutes into trans-institute kinds of programs that I think will make the role of the Director more prominent.

Senator KASSEBAUM. I certainly wish you well. I think that would be an important direction.

I would like to ask another question that stems from the announcement that was made last week by biomedical researchers at George Washington University about their research efforts to clone the human embryo. There was no NIH funding, I think, involved in that effort, but it does certainly raise many biomedical ethics questions. As you know, Senator Hatfield recently introduced legislation to create a standing ethics advisory board at Health and Human Services to address research and other biomedical issues.

Do you feel such a body is needed? How do you view this whole issue, which I think will continue to grow, and how will NIH deal with similar biomedical research efforts?

Dr. VARMUS. Well, you are raising two issues, Senator. One is the impact of the new results in embryo cloning, which as you know represent a relatively small advance scientifically, since this kind of duplication of embryos has been possible in animal species for some time, but of course, the new experiments focus our attention on them for possible application to human embryo cloning. And as you point out, NIH has not been funding such research for a variety of reasons. But nevertheless the research raises ethical issues that we need to front. The question is how we go about that mechanistically.

We have recently received permission from the Department to establish a subcommittee of the Director's advisory committee to look into the ethical implications of embryo research. There is the authority in law at the moment to establish ethical boards in response to specific issues. Whether we should have a standing ethics review board is a matter which I think I will leave to further consideration.

Senator KASSEBAUM. Thank you. I have gotten a notice that my time is about up. I was going to ask some questions on scientific integrity, but I would just like to close with a comment on your statement when you received the Nobel Prize you said it gave you the opportunity to speak out. You mentioned the indirect cost reimbursement issue and discussed and other areas which I think are all very important. I was particularly struck by your explanation of why basic research is essential to progress in so many other areas. I compliment you on recognizing the importance of basic research to gain an understanding in areas which are hard to comprehend and understand. We will certainly value your leadership at the National Institutes of Health.

Thank you.

Dr. VARMUS. Thank you.

The CHAIRMAN. Thank you very much.

Senator Simon.

Senator SIMON. Thank you, Mr. Chairman.

I have just a few observations. One is a personal situation that has been called to my attention from the State of Illinois. A girl by the name of Sarah Parsons lives in Barrington Hills, and she is one of 140 people in the Nation, mostly children, who have a

rare disease called urea cycle disorder, in which the ammonia level in the blood gradually rises until the brain is affected. The FDA has not yet approved a drug that has been developed by a pediatrician at Johns Hopkins, and those 140 people really depend on the NIH to continue to receive the drug until it does get FDA approval.

I do not expect you to know the answer on this, but if you——

Dr. VARMUS. I do know the answer, Senator.

Senator SIMON. You do know the answer. Good. I am impressed. Go ahead.

Dr. VARMUS. On October 18th, a letter was sent from Duane Alexander, the Director of the National Institutes of Child Health and Development, to the six Senators who signed the letter, asking that we provide some assurance that Dr. Brusselo, who heads these studies at Johns Hopkins, would continue to be supplied with phenyl butyrate, the drug in question. And although his funding status remains uncertain at this date, we will make every effort to assure that the children who are affected by this order will continue to receive the drug, which seems to be having beneficial effects.

Senator SIMON. I thank you very much.

Just three other areas——

Senator MIKULSKI. Didn't you love that?

Senator SIMON. I am impressed, I am impressed, yes. [Laughter.]

The whole area of mental health relative to the Nation's needs seems to me to be underfunded, and that brings up the whole question—and I do not expect you to answer it here and now—but I would appreciate a letter from you in terms of how you analyze what areas we go into in research. And you may not have a good feel for the answer of how we should go into this for another month or two, but I would be interested sometime in the next 60 days in receiving a reply from you on how we do that.

Dr. VARMUS. I will try to do that, Senator. I would be surprised if my mind is entirely clear on that issue in 60 days, because this is an extremely contentious issue that many of us have wrestled with for some time. I will make an effort.

Senator SIMON. And it is a very fundamental issue, obviously, for your position.

Second, I simply want to support an area that our colleague Senator Mikulski called our attention to, and I was stunned when I saw the facts, and that is that too often, we have not had a focus on research on women. For instance, there was a study on heart disease, and somehow, they just did not include women in that study.

Then, finally, there are at least a few who, rightfully or wrongfully, believe the Office of Research Integrity needs to be strengthened. I would just pass that along, and I am not certain whether that is accurate, but there seems to be enough substance to the concern that I think it should be examined.

I thank you very much. I think you are an extremely well-qualified person, and I am impressed that you are taking this on, and I wish you the best.

Thank you, Mr. Chairman.

Dr. VARMUS. Thank you, Senator.

The CHAIRMAN. Senator Coats.

Senator COATS. Thank you, Mr. Chairman.

Dr. Varmus, I want to add my congratulations to you also and tell you how much I enjoyed meeting with you in the office and how I wish you well as Director of NIH. But I am going to repeat some of the questions that I asked you earlier, just in two areas, if I could.

There is a book in Washington called "The Prune Book." Are you familiar with that book?

Dr. VARMUS. I have heard of it, Senator.

Senator COATS. That book describes certain positions available in Government. And the job of NIH Director is described, and I am quoting, as "one only for individuals with a substantial record in administering a major medical research or health care facility."

Now, I, like everybody else on this panel, am just tremendously impressed with you as an individual and with your background. You shared with me your love for the lab, and obviously, that love for the lab translated into phenomenal research work that led to the Nobel Prize, among many other distinguished awards.

How does an individual—an individual with a love for Renaissance literature and a love for the lab—face the daunting challenge of taking on the administration of a major national institution that is filled with political intrigue, daily inundated with requests from Senators and Congressmen in terms of suggestions as to how to manage, which people to fill which positions, how to direct funds, and so forth? In fact, even in your own testimony, I think you said that one of your goals was to support basic research and undirected funding.

To me, it almost seems comparable to taking a politician and saying you are going to get out of the camera lights and out of the public eye and go into the lab and research and come up with a discovery that is going to benefit mankind. I mean, I cannot imagine many politicians surviving in that atmosphere.

How do you make that transition, and how do you make it work for yourself and for the Institutes?

Dr. VARMUS. Let me try to respond, Senator. As an American Cancer Society research professor, I have been protected—prohibited from—taking on departmental chairmanships or deanships. But I am no stranger to many of the issues that confront the Director of NIH. I have wrestled with many of the substantive questions that an NIH administrator has to deal with, questions of research funding, questions of indirect cost, questions of research integrity, questions of appropriation of research funds. I have been in the thick of many of the battles and helped to effect resolution of some of these questions in the scientific community. I have been interested in a wide range of topics through my activities with the National Research Council of the National Academy of Sciences.

So although I have not had the titles, I have had positions of leadership within the scientific community that make me familiar with many of the things I will be asked to address.

Now, in the day-to-day management of NIH, I do have a big team, a team of accomplished deputies, institute directors. And my goal is to have excellent relations with them; when vacancies appear, to replace existing members of this team with excellent replacements, and to spend a good deal of time managing those as-

pects of the institution that I feel are in particular need of leadership.

I would point out in particular at the moment that the reconstitution of the Office of AIDS Research, the attention that needs to be paid to discrimination, the issues presented by the deterioration of the physical building in which is housed our Clinical Center and 40 percent of our laboratories, all of these things especially require my attention, and I will be giving it to them.

Senator COATS. You are going to need every bit of the obvious charm and impressive credentials that you possess, but you are also going to need a lot of steel to resist, I think, tremendous pressures in terms of how you administer the Institutes and how you direct the funding.

If I could just use my remaining time on that question of undirected funding, with all respect to my colleague from Illinois, who is not here—and I do not mean this in any disparagingly, because I would do the same thing—we are, for various reasons, personal family reasons or constituent requests, constantly attempting to direct the way in which NIH accomplishes its tasks and its funding. I cannot tell you how many times I have been on a plane flying back from Indiana, sitting next to someone on their way to NIH as part of an experimental program, and they turn to me and say, "There is a rumor that they are going to terminate the program at such-and-such a time. I know there are only nine of us in the world"—or 17, 150, whatever—"who are involved in this, but it is critical, and it is our only hope. And even though the odds are slim, I beg you, could you contact NIH to make sure they advance the program?"

We obviously want to be sensitive to that, and as I said, we bring our own personal family experiences in and say this is what the function ought to be. So we end up directing a lot of funds through our appropriations and authorization process. We direct a lot of funds and a lot of activities, perhaps in a way that you would not direct it as someone trying to look at the big picture.

You have expressed to me your interest in basic research, research that crosses the lines, has applications and benefits. How are you going to deal with that?

Dr. VARMUS. Well, there is no doubt that NIH has a balanced portfolio, Senator, like any other good managed concern, and we have many programs that address specific diseases, as we should. My concern is not that we continue to do those, because we should continue to pursue clinical research on specific disorders. But my concern is to protect, as the size of the research community increases and the funds for biomedical research fail to keep pace with the activities in our Institutes, that the basic research enterprise is also defended.

Senator COATS. It is going to be a tough job. I wish you well.

Thank you.

Dr. VARMUS. Thank you.

The CHAIRMAN. Senator Mikulski.

Senator MIKULSKI. Thank you very much, Mr. Chairman.

Dr. Varmus, before I go on to my questions for you, I would like to acknowledge to the committee someone in the audience who is very special, and that is Dr. Ruth Kirschstein, who has been the

acting Director of NIH during this bridge and also chaired the important initial task force on women's health at NIH. The committee should know that she has done an extremely yeoman's job over these last several months of helping NIH stay the course, helping Dr. Varmus with the transition, and I know she is ready to go back to her own scientific research. But we really owe her an enormous debt of gratitude for what she has done in the scientific community, the administrative work, and also the enormous debt that we the women feel to her.

Doctor, would you please stand up? You are so modest, but we want the committee to acknowledge you. In Montgomery County shopping malls, we are sometimes confused, but when they call me "Dr. Ruth," I do not know which one they are talking about. [Laughter.] And I feel Dr. Ruth feels the same way. So we do again thank you, Doctor.

Dr. VARMUS. Senator, may I make one comment? I appreciate your introducing Dr. Kirschstein; I feel that she has been neglected in these proceedings. And I want to point out that she is actually not returning to her institute but, to my great pleasure and gratitude, she is going to remain on as deputy Director of NIH and will be working hand-in-hand with me in the Director's office, and I am very grateful to her for that.

Senator MIKULSKI. Well, lucky for you, and a blessing for all of us.

Dr. VARMUS. Lucky for all of us, yes.

Senator MIKULSKI. Which then, of course, takes us to where you are, Dr. Varmus, and just a few quick comments. I would hope that the Senate would move to quick confirmation and that we do this certainly before the Thanksgiving break, not only because we want you on the job working with Dr. Kirschstein, but also the President is now in the process of getting ready to do his budget. And I felt that last year as we went through transition, budgets were late and often, because we were looking at the big picture in so many of the agencies, specific agencies like NIH and FDA and others that are really the critical agencies for our life sciences activities were not given the attention that they needed to be given.

I was concerned that NIH was really squeezed and perhaps not even understood. We want you on the scene working with Dr. Shalala and Dr. Lee and Leon Panetta, to make sure that the President's budget is really robust for NIH so that as it then comes over to the Budget Committee and subsequently to the Appropriations Committee, we can address these issues that my colleagues have outlined.

So that is why we want you on the job, and I think one of the things you need to sharpen is to make sure you pull out a green eyeshade and that lab coat of yours, because it probably is one of the most important areas of activity in your first 6 months.

But Dr. Varmus, could you share with us what your goals would be for NIH; could we get a fix on where you would like to be 6 months from now, where you would like NIH to be a year from now, and where you would like to see NIH 3 years from now, so that we can get a sense of your navigational chart, both in research and, as you said, in campus-related activities?

Dr. VARMUS. How much time do we have left, Senator?

Senator MIKULSKI. Well, let us take 6 months, 1 year, and 18 months.

Dr. VARMUS. There are some immediate things that we have in progress. We have several important searches, to find a director for the Office of AIDS Research, to find a new director for the Neurological Institute, to find a director for the Institute on Drug Abuse, and to find a director for the Clinical Center. These are four extremely important goals because of the major roles that each of these entities play within the NIH.

Second, we are undergoing a major reevaluation of the intramural research program, which has been mandated by language in the appropriations and reauthorization act, and I feel it is extremely important for our $1.2 billion investment in the intramural program to have an evaluation that allows us to make changes in that program that are effective.

Third, I would like to see the improvements in our attempt to establish strong principles for equal opportunity put in place. Dr. Kirschstein has made remarkable advances in this area in the last several months, and I would like to see those take effect so that we can decrease some of the charges of discrimination and sexual harassment that have plagued our campus.

And fourth, I would like to begin to address some of the encumbrances in our grant review system. Peer review is still a wonderful enterprise, and it is the right way to do grant review. Eighty-five percent of our budget, of course, is distributed to academic institutions around the country through the peer review process, but the peer review process has become encumbered in a variety of ways, and I am hoping to work closely with the Division of Research Grants to improve those processes.

Senator MIKULSKI. They are telling me I have 2 minutes left, which of course means I cannot cover that ground, and the best way to do that will be to visit you at NIH over the next several weeks, and I look ahead to doing that.

Finally, could you talk about extramural research? You mentioned the grant process, but also do you have any other goals either of what you want to do to stay the course on extramural research, or also new areas that you are considering? We have benefited from it so much in Maryland with Johns Hopkins.

Dr. VARMUS. As you know, Senator, I am still officially a member of the extramural community. That community is important to me, and I think it sometimes gets neglected in deliberations about the directions NIH is taking. And as we discussed a few days ago, one of the things I hope to do is to have a member of the office of the Director travelling around the country, looking for ideas from the extramural community that might have significant influence in the way we do business and the way we set goals.

This has been done over the years. I am sure you are all aware of the strategic planning process that received a great deal of publicity in the previous NIH administration, but in fact has been practiced by a number of institutes over the years. In that kind of process, investigators who work in academic institutions around the country are brought together to consider the plans for the next several years by any particular institute.

That has advantages, and I would like to see more of that process in effect. The products are often obsolete by the time they are published, but the process of thinking about directions often has a very direct effect on program planning, and I would like to see more of that go on.

Senator MIKULSKI. Thank you, Dr. Varmus. I do intend to visit NIH over the next several weeks, and we look forward to working with you on the issues our colleagues have outlined, and you know my particular interest in women's health, but in NIH itself.

Dr. VARMUS. It will be my pleasure to be your host.

Senator MIKULSKI. I am happy to have you abroad.

The CHAIRMAN. Senator Wellstone.

Senator WELLSTONE. Thank you, Mr. Chairman. I will be relatively brief.

First, I want to associate myself with some comments that have been made about my hope that we will move toward very quick confirmation.

One of the things that bothers me to no end—and I know that if Senator Harkin were here, he certainly would emphasize this point as well—is on the budget part, which Senator Mikulski spoke about. I think this is truly an area where it is myopic and so shortsighted if we do not really go to a necessary funding level. I mean, if you want to talk about an investment which pays for itself over and over and over again, not just in human terms, which is important enough, but also in economic terms, I think that is all about the NIH mission.

A disclaimer, Mr. Chairman, not conflict of interest, but disclaimer. I did mention to Dr. Varmus that both of my parents had Parkinson's disease, and therefore I have a very strong interest in this area. I have worked closely with Senator Harkin on this, and I just wanted to ask you one question on Parkinson's.

I am struggling—and I just wonder what your perspective is—to understand the lack of parity, if you will, in terms of absolute—as I look at spending on Parkinson's, both research and clinical dollars, it is the absolute lowest per patient, and yet we read about all sorts of real possibilities for cure. I wonder what your perspective is on the reason for that. This may apply to other neurological diseases as well. I have just looked carefully at the figures when it comes to Parkinson's. So I guess it is a) your perspective on the reason for that, and b) how you are thinking about how to begin to rectify this problem. We have talked about this some.

Dr. VARMUS. Yes. I think one of the difficulties, Senator, is identifying what money is actually being spent on Parkinson's disease. As you know, Parkinson's disease is a disease of the basil ganglia in which there is an apparent reduction in transmission of signalling through certain chemical mediators.

There is an enormous amount of research going on addressing the nature of those chemical mediators and the way in which nerve cells talk to each other. Not all of that research is labelled Parkinson's disease research, but nevertheless it has a direct impact on our understanding of the disease and could have a direct impact upon treatment.

Similarly, research that addresses general question of fetal tissue transplantation, although perhaps not labelled Parkinson's disease

research, also is likely to have a major effect on our ability to do fetal tissue transplantation in the treatment of that disorder.

I am prepared to look into this question for you in more detail, but I know from my readings of the activities of various institutes that Parkinson's disease is among the goals of basic research that addresses many aspects of behavior of nerve cells.

Senator WELLSTONE. Maybe what we can do is visit more on this. I have worked with any number of different organizations, Parkinson's Action Network and other groups, and people feel very strongly that there really has not been—for whatever reasons—a real parity. So I would just like to visit with you about that.

And remembering what Senator Simon said earlier, I do not do this in the spirit of wanting to pit one group of people who are struggling with a disease or a disability against another, which is something else that bothers me to no end about the budgets we are working within. But I do want to be a very strong advocate for this, and I would like to visit with you about it.

I have other questions that we can just talk about, but my last question is the extent to which you see the NIH mission moving more toward looking at some of the environmental causes of some of the diseases that afflict people in our country. I could begin to identify any number of them, but I think that has been an issue or point of contention.

Dr. VARMUS. As you know, we have a whole institute, the National Institute of Environmental Health Studies, that dedicates itself specifically to such questions. In the area of Parkinson's disease itself, both the Neurological Institute and the NIEHS do have programs that are addressing possible roles of environmental contaminants in Parkinson's disease. As you probably know, some years ago in California, there was a contaminant of an abused drug that induced in patients a syndrome that was extremely similar to classical Parkinson's disease. This allowed the development of an experimental model. It also drew attention to the possibility that noxious factors in the environment or in food might be potential candidates for causative agents for this disease.

So there are a number of grants that are currently active to support the pursuit of the possibility of environmental contaminants playing a role in Parkinson's disease itself.

Senator WELLSTONE. Or other diseases.

Dr. VARMUS. Yes, other diseases, of course. But I use that as an example.

Senator WELLSTONE. I thank you.

The CHAIRMAN. Just a very brief comment and a question. We would like to use the remainder of the time until the bells ring. I was listening to my friend Senator Coats talk about the steel backbone which you will need out there, and I think all of us would recognize that in talking about the qualifications to serve as the Director. As sort of an observer of people who run for the Presidency, if you are a Governor, they say you have no foreign policy experience; and if you are a Senator, you have no managerial experience. That sort of gets shaken out by the first primary, in any event, but it is inevitable in terms of the kinds of challenges that people face even in those offices, and yet through that process, I think all of us would feel that we have done very well.

Senator KASSEBAUM. And I would suggest, Mr. Chairman, we sometimes lack steel backbone here, as our constituents would say.

The CHAIRMAN. Just one additional question and then a very brief comment on the vacancies in the faculties out at NIH. As I understand, one of the great difficulties is that we have wrapped the NIH into the pay scales of Congress and the judiciary, and it is sort of, I am ashamed to say, a leverage to try to steep up the salaries of the Congress.

I would hope you could take a look at that and let us know about it. It is something that the institution is going to be reluctant to carve exceptions out for. In our committee, we have supported special foundations to raise private funds to augment those; we have put restrictions on the ability of individuals to supplement or augment their incomes. It is a tricky area, obviously, but it has been drawn to my attention at least that NIH has had more difficulty in attracting outside people to chair these various departments. I am interested in your recommendations in this area on ways for us to address this problem.

Finally, I particularly want to welcome your wife, Connie Casey, who was born in Boston. I know she has had a distinguished career as book editor of the San Jose Mercury News, book editor for the Los Angeles Times, and currently is the assistant editor of the Book World Sunday Book Section of the Washington Post. And her good father, Joseph E. Casey, is from Clinton, MA, an outstanding congressman who ran against Henry Cabot Lodge, and whose name has a ring, obviously, in terms of Massachusetts and our family. So the circle turns, and we are delighted to have her, and I think all of us in the State remember her father's service both in Massachusetts and to the Congress.

Dr. VARMUS. Thank you, Senator.

The CHAIRMAN. If there are no further comments or questions, I will say at this time that we have every intention of considering the nomination at the next executive session next week. We will do everything we can to expedite the nomination. I see no reasons why it should not be expedited. And I look forward, as I am sure the other members do, to supporting the nominee with great enthusiasm and pleasure.

We will leave the record open until next Tuesday for written questions and additional material supplied for the hearing record.

[Additional material supplied for the record follows:]

November 8, 1993

The Honorable Edward M. Kennedy
Chair, Senate Committee on Labor and Human Resources
U. S. Senate
Washington, D.C. 20510

Dear Ted,

I am enclosing four research articles that relate to the questions I addressed to Dr. Harold Varmus during his recent confirmation hearing and ask that they be included in the hearing record.

Thank you for your assistance.

Sincerely,

Paul

Paul David Wellstone
United States Senator

Parkinson's disease and exposure to agricultural work and pesticide chemicals

Karen M. Semchuk, PhD; Edgar J. Love, MD, PhD; and Robert G. Lee, MD, FRCP(C)

Article abstract—This population based case-control study of 130 Calgary residents with neurologist-confirmed idiopathic Parkinson's disease (PD) and 260 randomly selected age- and sex-matched community controls attempted to determine whether agricultural work or the occupational use of pesticide chemicals is associated with an increased risk for PD. We obtained by personal interviews lifetime occupational histories, including chemical exposure data, and analyzed the data using conditional logistic regression for matched sets. In the univariate analysis, a history of field crop farming, grain farming, herbicide use, or insecticide use resulted in a significantly increased crude estimate of the PD risk, and the data suggested a dose-response relation between the PD risk and the cumulative lifetime exposure to field crop farming and to grain farming. However, in the multivariate analysis, which controlled for potential confounding or interaction between the exposure variables, previous occupational herbicide use was consistently the only significant predictor of PD risk. These results support the hypothesis that the occupational use of herbicides is associated with an increased risk for PD.

NEUROLOGY 1992;42:1328-1335

Although Parkinson's disease (PD) is one of the most common adult neurologic disorders,[1] the etiology is still unknown. Interest in the possibility that an environmental toxin might be a causative factor[2,3] was rekindled by the discovery that MPTP (N-methyl-4 phenyl 1,2,3,6 tetrahydropyridine) produces a syndrome resembling PD.[4,5] Similarity in the chemical structure between the MPTP metabolite MPP+ and the herbicide paraquat triggered epidemiologic studies[6,7] that sought a possible relationship between PD, agricultural work, and the occupational use of pesticide chemicals. These studies have yielded conflicting results.

In a clinical study comparing patients with early-onset PD and controls,[8] the patients had a significant increase in the number of recessively inherited defects in hepatic hydroxylation. These hydroxylation mechanisms are responsible for metabolizing toxins. The possibility of a genetic susceptibility to toxins provided a plausible biologic mechanism linking agricultural work, pesticide exposure, and PD. Individuals with defective hydroxylation mechanisms might be less able to detoxify pesticide chemicals and hence might be more susceptible to their toxic effects. These susceptible individuals might have an increased likelihood of developing PD if directly exposed to these chemicals[9]—for example, through agricultural work. None of these hypotheses has previously been tested in a cohort study or in a population-based case-control study. The previous hospital-based case-control studies[10,11] have yielded conflicting results regarding the etiologic importance of rural environmental and agricultural exposures.

To further examine the hypothesized etiologic relationships between PD and exposure to rural environmental and occupational factors, we conducted a population-based case-control study of PD in the city of Calgary, Alberta, Canada. This study is the largest case-control study to test these hypotheses, and it is the first one that was population based. The initial portion of this study[12] found no significant increase in risk for PD associated with a history of rural living, farm living, or well-water drinking at any time during the first 45 years of life. We concluded that rural living, in itself, does not appear to be a strong risk factor for idiopathic PD. However, the possibility still exists that some

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Supported in part by the National Health Research and Development Program, Health and Welfare Canada, through a grant (no. 6609-1673-83) and through a National Health PhD Fellowship to Dr. Semchuk.

Received July 11, 1991. Accepted for publication in final form December 8, 1991.

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specific factors in the rural environment do play a role in the etiology of PD. The present study examines these issues further to look specifically at whether agricultural work (field crop farming, grain farming, market gardening, greenhouse work, or wood processing) or the occupational use of pesticide chemicals is associated with an increased risk for PD.

Methods. The cases were selected from a population-based case register of Calgary residents with neurologist-confirmed idiopathic PD. The methodology for the development of the case register and the eligibility criteria for the cases have been described in detail elsewhere.[27] Only living and nondemented idiopathic PD patients were recruited into the study by the attending physician; the decision regarding the presence or absence of dementia was a clinical decision of the attending physician. For each case, two individually matched (by sex and age, ± 2.5 years) community controls were selected by random-digit dialing.[28,29] Lifetime occupational histories, including information on work-related chemical exposures, were obtained by personal interviews by trained, experienced interviewers. The interviewer was not blind to the respondent's case-versus-control status. To minimize interviewer and respondent bias, the underlying hypotheses of the study were not revealed to either the interviewers or the respondents. For every job held for 1 month or longer, the interviewer recorded the start and stop dates and details about the nature of the work and any chemicals or compounds used on the job, including the dates on which the chemicals were first and last used. The respondents were asked specifically about previous work-related contacts with pesticide (herbicide, insecticide, fungicide) chemicals, including whether these chemicals were used on a grain farm, at a pulp or paper mill, in a commercial greenhouse, or on a market garden. Demographic data (date of birth, sex, marital status, education level, annual family income, ethnic background, and household size) and case profile data (age at symptom onset and age at diagnosis) were recorded.

Each completed occupational history was reviewed by one of the authors (K.M.S.), who coded the respondent's past occupational exposures (ie, exposed versus not exposed) to agricultural work and, more specifically, to field crop farming (grain farming and market gardening), commercial greenhouse work, wood processing (pulp processing and papermaking), and pesticide (herbicide, insecticide, and fungicide) chemicals. The variable agricultural work included all farming, horticultural, and wood processing occupations, except animal farmers, livestock workers, dairy farm workers, and poultry and hatchery workers. Exposures were coded for the first 15 years of life, for the period between the respondent's 16th and 55th birthdays, and for each 10-year age interval between the respondent's 16th and 65th birthdays. Cumulative lifetime exposures were coded for periods of increasing duration, by 10-year increments, with the respondent's 16th birthday as the reference date. Univariate and multiple conditional logistic regression analysis for matched sets[25] was used to estimate the relative risk (odds ratio) for developing PD associated with each exposure variable. In the univariate analysis, the crude, or unadjusted, relative risk of PD was estimated for each exposure variable separately. In the multivariate analysis, the adjusted relative risk of PD was estimated for each exposure variable, after controlling for potential confounding or interaction effects due to one or more of the other exposure variables of interest. All analyses used all of the available data, taking account of the full complement of controls selected for each case. While a missing value for the case or for both controls led to exclusion of the entire matched set, a missing value for one control meant that the number of controls in that set was reduced by one.[25] Potential gender differences were examined using stratified analysis techniques and by testing for interaction between each exposure variable and the variable sex. Statistical significance was indicated by an alpha level of 0.05 or less.

Results. One hundred thirty cases (75 men and 55 women) and 260 community controls (150 men and 110 women) were studied. The overall response rate was 88.4% for the cases and 75.8% for the controls. The respective case and control respondent and nonrespondent groups did not differ significantly in age, in the distribution of men and women and, for the cases, the age at diagnosis by a neurologist and the estimated duration of disease. The mean age (±1 SD) of the cases was 68.5 ± 11.5 years (range, 36.5 to 90.7 years) compared with 68.3 ± 11.3 years (range, 34.9 to 92.7 years) for the controls. The cases and the controls were comparable with respect to education level, annual family income, and ethnic background. However, a larger proportion of the cases were married (70.8% compared with 59.2% of the controls; $\chi^2_1 = 4.47$, $p = 0.034$) and fewer cases lived alone (9.2% compared with 30.8% of the controls; $\chi^2_1 = 14.25$, $p < 0.001$). For the cases, the mean age at symptom onset was 58.0 ± 12.2 years and the mean age at diagnosis by a neurologist, obtained by medical chart review, was 61.1 ± 12.4 years. The mean duration of disease, based on the age at diagnosis, was 7.8 ± 6.8 years. Eleven (9.1%) cases had been diagnosed before age 40. The male and the female cases did not differ significantly in age or in the estimated age at diagnosis. The estimated duration of disease, however, was somewhat longer for the women (9.4 ± 7.9 years compared with 6.7 ± 5.7 years for the men; $t = 2.12$; $df = 119$; $p = 0.036$).

Table 1 shows the exposure data for the cases and the controls and the crude PD risk estimates (odds ratios) associated with a history of occupational exposure to the various types of agricultural work and pesticide chemicals. Previous agricultural work and the occupational use of pesticides were reported by both the cases and the controls, and by both the male and the female respondents, although the male respondents were more likely to give a positive exposure history. No significant interactions were found between the variable sex and any of the exposure variables. At the univariate level, both previous agricultural work and previous occupational pesticide use were associated with significantly increased crude PD risk estimates. When the type of agricultural work or pesticide was considered, there was no significant increase in the crude PD risk estimate associated with previous exposure to field crop farming, grain

Table 1. Matched case-control sets, crude odds ratios for Parkinson's disease, and χ^2 values* by exposure variable: Calgary, AB, Canada, 1989

Exposure variable	Matched sets +++	++-/+-+/-++	+--	--+/-+-	---	+-	--	Crude odds ratio	χ^2	p value
Agricultural work	3	16	18	2	22	69	0	0	1.94 (1.12, 3.34)†	5.74	0.017
Field crop farming	1	15	17	4	21	72	0	0	1.68 (0.97, 2.91)	3.47	NS‡
Grain farming	0	11	15	3	22	78	1	0	1.51 (0.85, 2.66)	1.99	NS
Market gardening	0	1	6	0	4	119	0	1	2.68 (0.74, 9.68)	2.32	NS
Wood processing	0	0	3	0	2	125	0	0	3.00 (0.50, 17.95)	1.48	NS
Commercial greenhouse	0	0	0	0	3	127	0	0	ND§		
Pesticide use	1	8	23	4	17	77	0	0	2.25 (1.27, 3.99)	7.87	0.005
Herbicide use	0	5	12	0	9	99	0	2	3.06 (1.34, 7.00)	7.43	0.006
Insecticide use	0	1	16	0	16	96	0	0	2.05 (1.03, 4.07)	4.14	0.042
Fungicide use	1	1	14	1	16	94	0	0	1.63 (0.81, 3.29)	1.82	NS

* Goodness of fit χ^2 statistic with 1 df results of univariate conditional logistic regression for matched sets (cases and controls were individually matched by sex and age ± 2.5 years)
† 95% confidence limits.
‡ $p > 0.05$.
§ Odds ratio not determinable

The first symbol of each set represents the index case and the next 1 or 2 symbols, the matched controls. A "+" denotes a positive exposure. A "-" denotes a negative exposure

farming, market gardening, wood processing, or the occupational use of fungicides. However, a history of occupational herbicide use was associated with an estimated threefold increase in PD risk, which was statistically significant, and previous insecticide use resulted in an estimated twofold increase in risk, which was also statistically significant. The PD risk associated with commercial greenhouse work could not be estimated due to the small number of exposed respondents.

Table 2 presents the exposure data for the respondents and the crude odds ratios associated with a history of occupational exposure to the various types of agricultural work and pesticides during four 10-year exposure periods. The small number of respondents with a history of exposure before age 16 or after age 56 precluded estimation of the PD risk associated with exposures during these periods. There were significant increases in the crude PD risk estimates associated with a history of exposure to agricultural work during three periods of interest—ages 26 to 35 years, ages 36 to 45 years, and ages 46 to 55 years—and with a history of occupational use of pesticide chemicals between the ages of 26 and 35 years. When the type of agricultural work or pesticide was considered, statistically significant crude odds ratios were associated with a history of field crop farming, grain farming, or the occupational use of herbicides during three periods of interest—ages 26 to 35 years, ages 36 to 45 years, and ages 46 to 55 years—and with the occupational use of insecticides between the ages of 46 and 55 years. There was no significant increase in the crude PD risk estimate associated with a history of occupational use of fungicides during any of the periods of interest examined. The estimated PD risk associated with a history of market gardening, greenhouse work, or wood processing during these 10-year exposure periods could not be adequately assessed due to the insufficient number of exposed respondents.

In the univariate analysis, we also attempted to obtain crude estimates of the PD risk associated with increasing durations of cumulative lifetime occupational exposure to agricultural work and to pesticides (table 3). The results of this analysis suggest that there is a concomitant increase in PD risk with each 10-year increment in the cumulative lifetime exposure to agricultural work. While the risk associated with a lifetime history of 10 years' continuous exposure to agricultural work, ie, from ages 16 to 25 years, did not achieve statistical significance, 20 years of continuous exposure, ie, from ages 16 to 35 years, was associated with an estimated 2.5-fold increase in PD risk, which was statistically significant. Thirty years of continuous

Table 2. Matched case-control sets, crude odds ratios for Parkinson's disease, and χ^2 values* by exposure variable and exposure period: Calgary, AB, Canada, 1989

Exposure variable (ages exposed)	Matched sets										Crude odds ratio	95% confidence limits	χ^2	p value
	+++	+-+	+--	-++	-+-	---	+-	--	++	-+				
Agricultural work														
16-25 years	1	14	15	4	18	74	1	1	1	1	1 65	0 94, 2.92	3.02	NS
26-35 years	0	5	14	1	13	97	0	0	0	0	2 19	1.08, 4.45	4.74	0 029
36-45 years	0	2	11	0	8	109	0	0	0	0	2 93	1 20, 7.15	5.77	0 016
46-55 years	0	0	7	0	4	119	0	0	0	0	3.50	1.03, 11.96	4.20	0.040
Field crop farming														
16-25 years	1	13	14	4	19	75	1	1	1	1	1 49	0 84, 2.64	1 88	NS
26-35 years	0	5	13	1	10	101	0	0	0	0	2 59	1.20, 5.59	6 03	0 014
36-45 years	0	2	8	0	5	115	0	0	0	0	3.48	1.17, 10.34	5.37	0 020
46-55 years	0	0	6	0	3	121	0	0	0	0	4.00	1.00, 16.00	4.16	0 041
Grain farming														
16-25 years	0	10	13	3	19	76	2	4	0	2	1 39	0 77, 2.49	1 21	NS
26-35 years	0	4	10	1	9	104	1	1	0	0	2 31	1.02, 5.27	4 06	0 044
36-45 years	0	2	7	0	4	116	0	1	0	0	3 84	1 16, 12.70	5.31	0 021
46-55 years	0	0	5	0	2	122	0	1	0	0	5.00	0.97, 25.77	4.23	0.040
Pesticide use														
16-25 years	0	3	15	4	16	92	0	0	0	0	1 41	0.73, 2.73	1 04	NS
26-36 years	0	2	14	0	13	101	0	0	0	0	2 27	1 08, 4.76	4 72	0.030
36-45 years	0	3	11	0	11	105	0	0	0	0	2 21	0 99, 4.94	3.75	NS
46-55 years	0	1	11	0	11	106	0	0	0	0	2.07	0.91, 4.72	2.96	NS
Herbicide use														
16-25 years	0	2	4	0	7	111	0	2	0	0	1 40	0 46, 4 30	0 34	NS
26-35 years	0	2	9	0	4	109	0	2	0	0	4 82	1 51, 15 35	8 19	0 004
36-45 years	0	2	7	0	4	112	0	2	0	0	3 84	1.16, 12.70	5 31	0 021
46-55 years	0	1	7	0	3	114	0	2	0	0	4.88	1.28, 18.60	6.21	0.013
Insecticide use														
16-25 years	0	1	7	0	10	110	0	0	0	0	1 49	0.58, 3 81	0 67	NS
26-35 years	0	0	7	0	6	115	0	0	0	0	2 33	0.78, 6 94	2 30	NS
36-45 years	0	0	7	0	8	114	0	0	0	0	1 75	0.63, 4 83	1.14	NS
46-55 years	0	0	7	0	4	118	0	0	0	0	3.50	1.03, 11.96	4.20	0.040

* Goodness of fit χ^2 statistic with 1 *df*, results of univariate conditional logistic regression for matched sets (cases and controls were individually matched by sex and age ± 2 ½ years)

The first symbol of each set represents the index case and the next 1 or 2 symbols, the matched controls) A "+" denotes a positive exposure A "-" denotes a negative exposure

agricultural work exposure, ie, from ages 16 to 45 years, resulted in an estimated 3.5-fold increase in PD risk, which was also statistically significant. A similar trend was observed when the cumulative lifetime exposure to field crop farming or to grain farming was considered. Although the estimated twofold increase in PD risk associated with 20 years of continuous grain farming exposure did not achieve statistical significance, the estimated increase in risk associated with 30 years of continuous exposure to grain farming was more than fourfold, and statistically significant. The PD risk associated with more than 30 years of continuous agricultural exposure could not be adequately estimated due to the small number of respondents (six cases and three controls) with more than 30 years of exposure. Although the results of this analysis suggest a dose-response relation between the duration of cumulative lifetime exposure to agricultural work (field crop farming or grain farming) and PD risk, the analysis failed to demonstrate a similar dose-response relation with the cumulative lifetime occupational exposure to pesticides. However, few of the respondents (eight cases and 12 controls) gave a history of continuous occupational use of pesticides after age 25.

The data were submitted to multiple conditional logistic regression analysis to examine any potential confounding or interaction between the exposure variables. Table 4 shows the crude (unadjusted) odds ratio for PD associated with previous occupational herbicide use and the adjusted odds ratios for PD associated with previous occupational herbicide use after controlling for potential confounding due to the various other exposure variables. Consistently, regardless of which other variables were included in the logistic model, only the variable previous occupational herbicide use was associated with a statistically significant increased PD risk estimate. In all the logistic models examined,

Table 3. Matched case-control sets, crude odds ratios for Parkinson's disease, χ^2 values* and lifetime occupational exposure to agricultural work and pesticides: Calgary, AB, Canada, 1989

Exposure variable (exposed from age:)	Total no. of years exposed	Matched sets										Crude odds ratio	χ^2	p value
		+++	++→	+--	-++	-+→	---	+-	--	++	-+			
Agricultural work														
16 to 25 years	10	1	14	15	4	18	74	1	1	1	1	1.65 (0.94, 2.92)†	3.02	NS
16 to 35 years	20	0	5	11	1	9	104	0	0	0	0	2.45 (1.09, 6.54)	4.79	0.029
16 to 45 years	30	0	2	8	0	6	115	0	0	0	0	3.48 (1.17, 10.34)	5.37	0.020
Field crop farming														
16 to 25 years	10	1	13	14	4	19	76	1	1	1	1	1.49 (0.84, 2.64)	1.86	NS
16 to 35 years	20	0	5	10	1	6	106	0	0	0	0	2.50 (1.07, 5.87)	4.67	0.032
16 to 45 years	30	0	2	7	0	4	117	0	0	0	0	3.64 (1.16, 12.70)	5.31	0.021
Grain farming														
16 to 25 years	10	0	10	13	3	19	76	2	4	0	2	1.39 (0.77, 2.49)	1.21	NS
16 to 35 years	20	0	4	8	1	7	108	1	1	0	0	2.39 (0.96, 5.93)	3.62	NS
16 to 45 years	30	0	2	6	0	3	118	0	1	0	0	4.44 (1.15, 17.09)	5.33	0.021
Pesticide use														
16 to 25 years	10	0	3	15	4	16	92	0	0	0	0	1.41 (0.73, 2.73)	1.04	NS
16 to 35 years	20	0	2	6	0	10	112	0	0	0	0	1.38 (0.53, 3.57)	0.43	NS
16 to 45 years	30	0	1	3	0	6	118	0	0	0	0	0.88 (0.26, 2.99)	0.04	NS

* Goodness of fit χ^2 statistic with 1 d.f., results of univariate conditional logistic regression for matched sets (cases and controls were individually matched by sex and age ± 2.5 years)
† 95% confidence limits

The first symbol of each set represents the index case and the next 1 or 2 symbols, the matched control(s). A "+" denotes a positive exposure. A "-" denotes a negative exposure.

having a history of occupational herbicide use resulted in a significantly increased PD risk of approximately threefold. Based on the exposure level in the community control sample and the crude PD risk estimate of 3.06 (table 1), the estimated population attributable risk (ie, percent of PD cases in this study that can be explained by the exposure factor)[25] associated with previous occupational herbicide use is 10%. After adjusting for previous occupational herbicide use, there was no significant increase in PD risk associated with a history of agricultural work (adjusted odds ratio, 1.56; 95% confidence limits, 0.85 and 2.85), field crop farming (adjusted odds ratio, 1.23; 95% confidence limits, 0.67 to 2.28), grain farming (adjusted odds ratio, 0.97; 95% confidence limits, 0.49 to 1.92), or occupational insecticide use (adjusted odds ratio, 1.48; 95% confidence limits, 0.68 to 3.24). There were no significant interactions between any of the exposure variables examined, or between any of the exposure variables and the variable sex.

Following the multivariate analysis, we reviewed the interview records of the herbicide-exposed cases to identify the specific herbicide compounds they had used. Among those cases (41%) who could recall the chemical or trade names of the herbicides used, all but one had used compounds in the chlorophenoxy and thiocarbamate chemical groups exclusively, and primarily in grain farming. One case reported having worked with the pyridylium compound paraquat, between the ages of 26 and 31 years. He was the only herbicide-exposed case whose onset of symptoms occurred before the age of 40.

Discussion. The results of the univariate conditional logistic regression analysis suggest that individuals with a history of agricultural work (field crop farming or grain farming) or the occupational use of pesticide chemicals (herbicides or insecticides) have an increased PD risk. The univariate data also suggest a dose-response relation between the duration of cumulative lifetime exposure to agricultural work and PD risk. However, the

Table 4. Adjusted odds ratios* for Parkinson's disease and herbicide use: Calgary, AB, Canada, 1989

	χ^{2}†	χ^{2}‡	*df*	Odds ratio	95% confidence limits	*p* value
Crude: Herbicide use	7 43			3.06	1.34, 7.00	0.006
Adjusted for:						
Insecticide use		0 98	1	2.60	1.07, 6 32	0 036
Fungicide use		1 27	1	3.72	1.43, 9 65	0 007
Agricultural work		2 04	1	2.46	1.03, 5 94	0 042
Crop farming		0 44	1	2.75	1.14, 6.64	0 025
Grain farming		0 32	1	3.17	1.27, 7 93	0 014
Market gardening		2 76	1	3.16	1 38, 7 22	0 006
Wood processing		1 87	1	3.17	1.36, 7 29	0 007
Insecticide use + fungicide use		2 18	2	3.22	1 19, 8 74	0 022
Insecticide use + fungicide use + agricultural work		5 05	3	2 91	1.06, 8 01	0 039
Insecticide use + fungicide use + crop farming		2 94	3	3 02	1.10, 8 29	0 033
Insecticide use + fungicide use + grain farming		2 39	3	3.14	1.13, 8 74	0 028
Insecticide use + fungicide use + market gardening		4 75	3	3 21	1 18, 8 68	0 022
Insecticide use + fungicide use + wood processing		3 82	3	3.39	1.24, 9 24	0.017

* Results of multiple conditional logistic regression for matched case-cases and controls were individually matched by sex and age ± 2 5 years
† Goodness of fit chi-square statistic with 1 *df*
‡ Improvement in chi square statistic by adding the variable(s) to the single-variable logistic model with the independent variable "herbicide use"

Table 5. Previous case-control studies of Parkinson's disease, agricultural work, and pesticide use

Location	Cases/ controls	Exposure variable	Odds ratio
Sweden*	91/78	Agricultural chemicals	NS†
		Mercury	NS
		Organic solvents	NS
China*	100/200	Chemical manufacturing	2 18‡
		Wheat growing	0 69‡
		Corn growing	NS
		Fruit growing	NS
		Soybean growing	NS
		Rice growing	NS
		Paper mills	NS
Hong Kong*	35/105	Farming 1 20 years	NS
		Farming >20 years	5 2‡
		Herbicide/pesticide use	3 6‡
Kansas*	150/150	Farming	NS
		Herbicide/pesticide use	NS
Quebec*	42/84	Farming	NS
		Pesticide use	NS
Illinois*	78/78	Farming	3 0‡
		Herbicide/pesticide use	NS
New Jersey*	106/106	Farming	NS
		Herbicide/pesticide use	3 0‡
British Columbia*	57/122	Orchard work	4 45‡
		Planer mill work	NS
		Chemical spraying	5 0‡

* $p < 0.01$
† $p < 0.01$
‡ Not statistically significant $p > 0.05$

results of the multiple conditional logistic regression analysis, which controlled for potential confounding and interaction between the exposure variables, implicate only the occupational use of herbicides as a significant risk factor for PD. Consistently, having a history of occupational herbicide use resulted in a significantly increased PD risk of about threefold, regardless of which other exposure variables were included in the logistic model. The estimated increase in risk was similar for exposed male respondents and for exposed female respondents, although the male respondents were more likely to give a positive exposure history.

Interestingly, after adjusting for previous occupational herbicide use, the significant increase in PD risk associated with agricultural work (field crop farming or grain farming) and with occupational insecticide use in the univariate analysis disappeared, indicating that the increased risk associated with these exposures in the univariate analysis was likely due to the effects of herbicide exposures associated with the work and with the use of insecticides. These data indicate that while agricultural work (field crop farming or grain farming) or occupational insecticide use might serve as a risk marker for identifying potentially high-exposure, high-risk groups, such as grain farmers, direct contact with herbicides, afforded by regular occupational handling of these chemicals, should be regarded as a risk factor for PD.

Despite the consistent finding, in both the univariate and the multivariate analyses, of a significantly increased PD risk associated with occupational herbicide use, the analysis failed to demonstrate a dose-response relation between the duration of cumulative lifetime occupational exposure to herbicides and PD risk. The possibility of a critical age for exposure to herbicide chemicals is unlikely, as an increased PD risk was associated with the occupational use of herbicides during three different 10-year age intervals. It is suspected, however, that this study did not have adequate statistical

power to demonstrate a dose-effect relation with the cumulative lifetime occupational herbicide use for the following reasons: (1) the small number of respondents with a history of occupational use of herbicides, (2) the sharp decline in the number of respondents who were exposed after age 25, and hence, (3) the small number of respondents with more than 10 years of continuous occupational use of herbicides.

Due to the inability of the majority (59%) of the exposed cases to recall the names of the herbicides they had used, we were unable to estimate the PD risk associated with occupational exposure to specific herbicide compounds or chemical groups. All but one of the cases who could name the herbicides used had used compounds in the chlorophenoxy or thiocarbamate chemical groups. Interestingly, the one individual who recalled having worked only with paraquat was also the only herbicide-exposed case to experience an early symptom onset—that is, before the age of 40.

At present, there is no conclusive empirical evidence to implicate any of the chlorophenoxy, thiocarbamate, or pyridylium compounds in the etiology of PD.[26,27] However, there have been case reports of early-onset parkinsonism in a farmer with extensive pesticide exposure, including chlorophenoxy compounds,[28] in a man who had previously worked in a chemical plant making petroleum derivatives and pesticides,[29] and in a citrus farmer who had worked with paraquat for 15 years.[30] There also have been reports of cerebral changes in cases of lethal paraquat poisoning.[31,32] An epidemiologic study of workers using paraquat, however, failed to reveal any cases of parkinsonism,[33] and there are no animal models of paraquat-induced parkinsonism. Most recently, there has been a case report of a 72-year-old farmer in Italy who developed a severe parkinsonian syndrome several days after having sustained about a 10-minute exposure of the hands to a 10% aqueous solution of the contact herbicide diquat dibromide.[34]

Before the start of this study, there was only one published case-control investigation[22] of the hypothesized relationship between agricultural exposures and PD risk. In Sweden, Ohlson and Hagstedt[22] found that PD patients and hospital controls did not differ significantly in their occupational exposures to various organic solvents used in agriculture and to mercury. However, the sample studied was small, and the nonsignificant results might have been explained by inadequate statistical power or any of a number of biases: selection bias (hospital-based sample), recall bias, as more than one half of the data was obtained from surrogates of deceased patients (50.6%) and controls (56.0%); or biased sampling toward the selection of patients with early-onset PD, as only cases diagnosed between the ages of 35 and 69 were included.

Just before completion of the present study, reports were published of case-control studies of PD and agricultural exposures in Chinese,[35,36] American,[37,38,39] and Canadian[40,41] residents. However, none of these case-control studies was population based and their results are conflicting. The results of these studies are summarized in table 5. The findings of our population-based study concur with the results of five of the previous hospital-based case-control studies.[35,36,38,39,41]

In conclusion, this population-based case-control study did not have the statistical power to demonstrate a dose-response relation between the duration of lifetime occupational exposure to herbicides and the estimated PD risk. However, the biologic plausibility of the hypothesized etiologic relation between herbicide exposure and PD, and the consistency of the evidence in this study with previous case reports and hospital-based case-control studies of very different populations, support the hypothesis that the occupational use of herbicides is associated with an increased risk for PD. Caution should be taken, however, in generalizing the results of this study to PD cases with dementia, since it is not known to what extent the distribution of exposures differed between the excluded demented cases and the cases studied.

If exposure to herbicide chemicals does play an important role in the etiology of PD, one would expect to see a concomitant increase in the incidence of the disease with increasing use of these chemicals over time. Herbicides have been used extensively in crop production in North America, including western Canada, since about 1945.[42] However, a prospective study of the population of Rochester, Minnesota, from 1945 through 1979, has found the incidence of PD to be stable in this population over this 35-year period.[43]

While the observed stability in the incidence of PD subsequent to the introduction of herbicides in North America might indicate the absence of an etiologic association between herbicide exposure and PD, it might also be reflective of a multifactorial etiology. This conclusion is suggested by the finding that although occupational herbicide use was consistently associated with a significantly increased PD risk of approximately threefold, previous occupational herbicide use explained only an estimated 10% of the cases in this study, the remaining 90% being explained by other not-yet-known factors. The apparent stability in the PD disease incidence over time might also be an artifact. There are no population-based estimates of the incidence of PD for the period prior to the use of pesticides with which to compare the more recent estimates.[1] Alternately, the stable PD incidence rates might reflect the effects of a disappearing causal agent. This hypothesis has been proposed to explain the declining annual incidence of amyotrophic lateral sclerosis/parkinsonism-dementia observed on the small island of Guam.[44] The majority of the herbicide-exposed respondents in this study were older and had used herbicides long before the use of personal protective devices became commonplace. It was not uncommon for

these respondents to report that they had mixed these chemicals with their bare hands on several occasions. We suspect, therefore, that the respondents in this study had sustained much higher occupational herbicide exposures than do farmers and others working with these chemicals today. Thus, safer pesticide handling and application practices should have decreased the personal exposure levels of those working with these chemicals over time and might, conceivably, lead to a decreased incidence of PD at some future date. It may be too soon to see the effects of this yet.

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Environmental antecedents of young-onset Parkinson's disease

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Article abstract—We conducted an exploratory study of young-onset Parkinson's disease (YOPD) to examine occupational and environmental factors associated with disease risk. This case-control study included 63 YOPD patients (diagnosis on or before age 50); controls (n = 68) were diagnosed with rheumatoid arthritis. Crude odds ratios (ORs) were computed to identify exposure variables for logistic regression analyses. After controlling for the variables of race, educational level, sex, age, age at diagnosis, and family history of Parkinson's disease (PD), PD was positively associated with insecticide exposure (OR = 5.75, $p < 0.001$), past residency in a fumigated house (OR = 5.25, $p = 0.046$), herbicide exposure (OR = 3.22, $p = 0.0331$), rural residency at time of diagnosis (OR = 2.72, $p = 0.027$), and nuts and seed eating 10 years before diagnosis (OR = 1.49, $p = 0.021$). PD was inversely associated with cigarette smoking at 5 years (OR = 0.50, $p = 0.027$), 10 years (OR = 0.43, $p = 0.012$), and 15 years (OR = 0.37, $p = 0.005$) before diagnosis, farm residency (OR = 0.38, $p = 0.018$), and exposure to dimethyl sulfoxide (OR = 0.10, $p < 0.001$). These findings are consistent with hypotheses linking PD to exposure to pesticide agents.

NEUROLOGY 1993;43:1150-1158

Over 300 studies have explored the relationship between idiopathic Parkinson's disease (PD) and exogenous chemicals.[1] Both basic and applied research point to an etiology in which multiple risk factors may be necessary for the expression of disease. Ecologic studies show fairly uniform rates of disease when PD prevalence is measured at a national, state, or province level. However, more detailed studies demonstrate these seemingly uniform patterns of prevalence may conceal wide variations of disease occurrence at the local or county level. Factors associated with PD occurrence in analytic epidemiology studies include rural residency,[2-11] potable water source from a private well,[4,7,9,11-13] participation in farming,[2,5,12,14-16,21] and exposure to pesticide products.[2,13,14,17-21] In addition to these positive associations, studies from diverse geographic and cultural settings have yielded fairly consistent findings of an inverse association between PD and cigarette smoking.[5,22,29] Despite these clues, specific agents have yet to be identified, and the etiology of PD is unknown.

To examine exogenous risks for PD, we undertook a hypothesis-generating study of chemical exposures in the occupational and nonoccupational environment. The purpose of the study was twofold: (1) to conduct a broad-based examination of chemical risks previously identified in the PD literature, and (2) to formulate sets of PD predictors through the use of multivariate analysis techniques. The study methodology was designed to facilitate a comprehensive search for potential triggers of young-onset Parkinson's disease (YOPD) in multiple exposure categories (ie, occupational, recreation and hobbies, nutritional). Although such an approach is vulnerable to bias in the quantification of exposures, it provides some indication, however crude, of the nature of chemical exposures that people encounter during their lifetime. Because current estimates of the induction period of PD range from months to decades,[30] obtaining the best possible lifetime exposure history was a primary goal of the study.

Two of the more unique features of the design included the exclusive study of young- versus older-onset PD patients and the selection of patients with rheumatoid arthritis (RA) as the control group. The decision to focus on young-onset patients was based on the premise that patients who present with PD early in life may have been exposed to a greater dose of a putative agent (or agents) than those who develop the disorder later.

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Supported in part by the National Research Service Award #1 F31 NR06431, National Center for Nursing Research, NIH. P.S.S. was supported in part by NIH grant NS 19611.

Received August 10, 1992. Accepted for publication in final form October 22, 1992.

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Second, the validity of exposure data may be greater in a cohort of young- versus older-onset patients; simply stated, they have a shorter life to recall. Finally, young-onset patients are more likely than older-onset patients to have living parents and siblings; these family members are critical for the recall of early life exposures. Rajput et al[4] were among the first researchers to target young-onset patients for analytic epidemiologic studies of PD. Since that time, YOPD has come to be defined as onset of PD signs between the ages of 21 and 40.[31] For the present study, a somewhat less restrictive definition of YOPD was adopted; patients were considered eligible for study if they received a diagnosis of PD on or before the age of 50.

The use of RA patients as the control group addressed the issue of recall bias, a primary threat to the validity of case-control and other retrospective designs. Patients who are afflicted with chronic diseases are more likely than the general population to have spent time thinking about their exposure history. In the present study, the selective recall between subject and control groups was "balanced" by the selection of controls with another chronic disease of unknown etiology, RA. Like PD, the RA patient population is characterized by wide variations in functional impairment and frequent problems with handwriting and fine motor skills. Furthermore, treatment in both disorders is generally symptomatic rather than curative.

Methods. *Subjects.* Subjects included 63 persons diagnosed with idiopathic PD (ICD-9 code 332.0) on or before the age of 50 with current residence in Oregon or Washington; controls included 68 persons diagnosed with RA between the ages of 29 and 51 living within the same catchment area. Controls were frequency matched with subjects for sex, year of birth, and year of diagnosis. Both subjects and controls were asked to respond to a mailed exposure history questionnaire. Inclusion criteria for PD were adapted from those specified by Ward and Gibb[32] and included the following: (1) progressive disorder; (2) presence of at least two of the following—tremor, rigidity, bradykinesia, postural instability; (3) absence of clinical features characteristic of alternate diagnoses; (4) absence of significant cognitive impairment; and (5) absence of etiology known to cause secondary parkinsonism. Diagnosis with idiopathic PD on or before age 50 and inclusion criteria status were verified for each patient through telephone or letter contact with the primary neurologist.

Measurement of exposure. The literature addressing the etiology of PD and existing survey instruments were reviewed to identify relevant topic areas for the exposure history questionnaire. Additional exposure categories for the questionnaire were generated through a series of open-ended interviews with 14 patients diagnosed with young-onset neurodegenerative diseases. The purpose of these interviews was to generate the broadest possible list of potential triggers of disease. During the interview process, patients were asked to reconstruct their employment and exposure histories. Interview transcripts were reviewed by the authors to generate additional exposure categories deemed biologically plausible for the induction of PD. These interviews also provided information about subjects' recall and level of comprehension for chemical exposures. The questionnaire was edited for clarity and use of jargon compatible with the general population; in the final step, it was subjected to review by epidemiologists from the Oregon Health Division and members of the Parkinson's Epidemiology Research Committee, an interdisciplinary group consisting of experts in PD and related neurodegenerative disorders. Content areas addressed by the questionnaire included (1) medical/surgical/dental history; (2) residential/travel history; (3) nutritional history/food preferences; (4) exposure to insecticides, herbicides, fungicides, and rodenticides; (5) chronology of occupations and industries; (6) exposure to prevalent occupational neurotoxins; (7) exposure to lead, mercury, and manganese; (8) prescription and over-the-counter medication use; (9) recreational drug exposure; (10) use of personal hygiene products; and (11) exposure to natural, organic, and homeopathic remedies. This paper addresses findings that relate to the first four categories of exposure. To reduce bias in the classification of exposures, exposure was defined as (1) "direct exposure to dust or fumes from the substance," (2) "prolonged and direct skin contact with the substance," or (3) "ate or drank the substance."

Analyses were performed using CRUNCH and CRUNCH4 statistical packages. Data analysis of the exposure variables involved a three-step process; first, all variables were subjected to bivariate analyses (crude odds ratios [ORs], t tests) to identify potential risk factors to be included in multivariate analyses. In step two, all variables for which $\alpha < 0.10$ in the bivariate analyses were subjected to multivariate analyses using unconditional logistic regression techniques; ORs for each risk factor were adjusted for potential confounding variables (age, age at diagnosis, sex, educational level, race, and family history of PD). In the final step of the analysis, models including two or more risk factors were formulated to identify sets of independent predictors of PD. In the multivariate analyses, statistical significance was indicated by an α level of 0.05 or less. All p values reported are two-tailed.

Reliability and validity of questionnaire. An important phase in the study design focused on establishing preliminary evidence of the questionnaire's reliability and validity. Test-retest reliability of the questionnaire was assessed by administering an abridged version of the questionnaire to a small group ($n = 8$) of patients. Second administration occurred 1 month following first administration. Six of 21 categorical variables showed no variance from time one to time two. Of the remaining 15 variables, 11 had kappa values greater than 0.75, a value interpreted by Landis and Koch[33] to represent excellent agreement beyond chance. The validity of subjects' responses was assessed by checking questionnaire data against medical record and employment data. Past job titles and descriptions were verified through telephone calls to employers. History of fracture, soft tissue injury, and surgery was verified through review of medical record data.

There was strong agreement between subjects and employers in regard to job title. Some semantic differences in job title were noted across data sources (ie, subject listed job title as "education manager," while employer listed job title as "education specialist"); however, these small differences did not affect coding of job titles as per the census code occupational classification system. There was 80% agreement (within 2 years) between subject and employer listings for dates of employment.

Concordance between questionnaire and medical record data in regard to past surgery, fracture, and injury was 92%.

Subject recruitment. Multiple strategies for subject recruitment were employed to provide an opportunity for all eligible PD patients living in Oregon and Washington to participate. Both physician- and patient-based referrals were solicited. For the former, a letter requesting referrals was sent from two of the investigators (P.S.S. and J.G.N.) to all Oregon and Washington neurologists listed in the directory of the American Academy of Neurology. In addition, patients were recruited directly through contacts with YOPD support groups affiliated with Oregon Health Sciences University (OHSU) and University of Washington. Parallel activities were set up for the recruitment of controls; activities included a recruitment letter to rheumatologists in Oregon and southern Washington and contacts with the OHSU rheumatology clinic and rheumatology support groups. Additional controls were solicited from a registry of Kaiser Permanente arthritis patients; this referral source permitted ease of subject-control matching.

Response rate/reasons for nonresponse. The exposure history questionnaire was mailed to 100 PD patients. Of those 100 patients, 23 did not respond, five patients returned a blank questionnaire, two patients were too ill to participate, and one patient with English as a nonprimary language declined the option of participating through a translator. Four additional patients were excluded from the analysis because of diagnosis after the age of 50. Two patients failed to meet the study inclusion criteria; one of these had signs indicative of multisystem atrophy that had become apparent during the past few months. The second patient had been employed in the law enforcement field and had a rapid onset of extrapyramidal signs following the burning of illicit drugs in a police drug-disposal unit; the physician concluded that drug-induced parkinsonism could not be ruled out.

Questionnaires were mailed to 185 potential controls. Seven questionnaires were returned with an incorrect address; efforts to obtain current address information were unsuccessful. Of the 179 potential controls who received questionnaires, 11 failed to meet the study inclusion criteria because of diagnosis later than age 50 or other reasons. Final response rates for subjects and control groups meeting inclusion criteria were as follows: of the 92 potential PD subjects, 63 chose to participate in the study, yielding a response rate of 69%. Sixty-eight of 168 potential controls returned completed questionnaires, yielding a response rate of 41%.

There were no statistically significant differences ($p < 0.05$) on t tests comparing subject and control groups on the continuous variables of age, age at diagnosis, and duration of disease. Chi-square tests run on the categorical variables of sex and race revealed no significant differences between PD subjects and controls for proportion of male sex ($p = 0.216$). However, there was a significant difference between the two groups in regard to proportion of white versus nonwhite participants ($p = 0.018$). Since the factors of age, age at diagnosis, educational level, sex, and race can influence either the occurrence of PD or the recall of potential etiologic exposures, these variables were controlled in subsequent statistical analyses. The section on logistic regression analysis outlines procedures that relate to the statistical control of these variables.

Results. *Demographic characteristics of sample.* Age at diagnosis for PD subjects ranged from 25 to 50 years with a mean of 41.1 years, compared with 29 to 50 years and 41.2 years respectively for the control group. Sixty-three percent of PD subjects were men compared with 52% of controls. Current age of subjects with PD (n = 63) ranged from 35 to 72 years, with a mean of 49.0 years. Controls (n = 68) ranged in age from 35 to 71 years, with a mean of 50.8 years. One PD subject was Native American and the remainder were white. This distribution was more varied in the control group, which consisted of 87% white, 8% Native American, 3% Asian/Pacific Islander, and 3% Hispanic.

Analysis of exposure data. The study's findings are presented according to the following exposure categories: medical/surgical conditions, patterns of rural living, exposure to pesticide products, and patterns of food, beverage, and cigarette consumption. Although not an environmental exposure, family history of neurodegenerative disease is included in this section because positive family history of one or more of these disorders has been associated with increased disease risk.

Family history of cancer, arthritis, and neurodegenerative disease. Subjects and controls were asked about their family history of cancer, RA, and neurodegenerative disease. "Family" was operationally defined as parents, grandparents, siblings, aunts/uncles, and first cousins related to the subject by blood. Using these criteria, 16 subjects (25.4%) had at least one relative with PD, compared with seven controls (10.3%); this difference was statistically significant ($p = 0.037$). For those PD subjects who had a positive family history, 14 subjects had one affected relative, one subject had two affected relatives, and one subject had three affected relatives. Those affected were most likely to be the subject's aunt or uncle (n = 8), followed by a parent (n = 6), sibling (n = 3), or grandparent (n = 2). There were no significant differences between subjects and controls for family history of Alzheimer's disease (19.1% subjects, 8.8% controls, $p = 0.127$), ALS (3.2% subjects, 4.4% controls, $p = 1.0$), RA (31.8% subjects, 48.5% controls, $p = 0.074$), or cancer (74.6% subjects, 67.7% controls, $p = 0.443$).

Medical-surgical conditions. Because of previously reported associations between parkinsonism and general trauma, head trauma, and encephalitis, PD subjects and controls were queried regarding their medical-surgical histories. Subjects were significantly less likely than controls to have ever used dimethyl sulfoxide (DMSO); the adjusted OR for this variable was 0.10 ($p < 0.001$). No significant associations were noted for variables labeled: "ever had a fracture?" (crude OR = 1.65, $p = 0.162$), "ever been knocked out from a blow to the head?" (crude OR = 1.72, $p = 0.174$), "ever had an infection of the brain?" (crude OR = 1.05, $p = 1.000$), and "ever received a strong electrical shock?" (crude OR = 0.47, $p = 0.247$).

Patterns of farm and rural residency. The

adjusted OR for "ever lived or worked on a farm?" was 0.38 (p = 0.018). However, further analyses of these data revealed differences between PD subjects and controls in relation to duration of residency. PD subjects with a history of farm employment or residency had a mean of 10.6 years of exposure compared with controls, who had a mean of 6.4 years (t test p = 0.101). Similar patterns of exposure were noted in data addressing rural residency (defined as residence in a community of 10,000 or less). The crude OR for rural residency at birth was statistically significant (crude OR = 0.48, p = 0.49); however, after adjusting for potential confounders this association was no longer significant (adjusted OR = 0.44, p = 0.062). In contrast, crude ORs for rural residency at all time points following birth were greater than 1, although significant only at time of diagnosis (crude OR = 2.59, p = 0.029; adjusted OR = 2.72, p = 0.027). Table 1 lists percentage of subjects exposed, percentage of controls exposed, and crude ORs for rural residency at six time points. Use of potable water from a private well was not significant for any of the four recorded times (5, 10, 15, and 20 years before diagnosis). Crude ORs for this exposure category ranged from 0.51 (p = 0.494) to 1.21 (p = 0.797).

Pesticide-related exposures. Crude ORs were computed for six variables that represented opportunities for exposure to pesticide products. Subjects/controls were asked to respond affirmatively if they were directly exposed to the substance in question more than 10 times a year in any year.

Table 1. Number of subjects/controls exposed, percentage subjects/controls exposed, and crude odds ratios for residency in a town of 10,000 or less at six time points

Variable	Subjects responding "yes"	Controls responding "yes"	Crude odds ratio	p
Rural residence (RR) at birth	20 (32)	33 (50)	0.48	0.049
RR 20 years before diagnosis	19 (31)	16 (24)	1.34	0.433
RR 15 years before diagnosis	21 (34)	13 (19)	2.26	0.068
RR 10 years before diagnosis	21 (33)	15 (23)	1.67	0.240
RR 5 years before diagnosis	20 (33)	17 (29)	1.41	0.432
RR at time of diagnosis	23 (37)	12 (18)	2.59	0.029

To define terms specifically for subjects/controls, herbicides were defined as "products that kill plants and weeds," fungicides as "products used to control blight and mildew," and rodenticides as "rat and mole poison." Fumigation was operationally defined as a "tent placed over the home and insecticides applied." In the bivariate analyses, PD was found to be significantly associated (p < 0.10) with three variables representing exposure to different categories of pesticides; these variables included exposure to herbicides (crude OR = 3.46, p = 0.011), past residency in a fumigated house (crude OR = 3.29, p = 0.068), and exposure to insecticides (crude OR = 4.04, p = 0.002). Crude ORs for fungicide exposure (OR = 1.50, p = 0.742), rodenticide exposure (OR = 2.42, p = 0.618), and "ever lived within ¼ mile of agricultural spraying?" (OR = 1.99, p = 0.106) were not significant.

Those variables significant in the bivariate analyses were subjected to multivariate analysis using logistic regression techniques. Because of the hypothesis-generating nature of the study, the variable representing "ever lived within ¼ mile of agricultural spraying?" was included in the multivariate analyses, although it was not statistically significant (crude OR = 1.99, p = 0.106). After adjusting for the variables of age, age at diagnosis, family history of PD, sex, race, and educational level, the OR for insecticide exposure was 5.75 (p < 0.001). No temporal patterns were observed for either subjects or controls; time of exposure ranged from 1 to 46 years before diagnosis for PD subjects and 0 to 38 years before diagnosis for controls. PD subjects were exposed to insecticides more frequently than controls (mean for subjects = 27.8 times/year; control mean = 10.8 times/year). ORs for herbicide exposure and past residency in a fumigated house were 3.22 (p = 0.033) and 5.25 (p = 0.046) respectively. The adjusted OR for "ever lived within ¼ mile of agricultural spraying?" was 1.99 (p = 0.099). Logistic regression analyses comparing the relative strength of association between insecticide and herbicide exposure produced adjusted ORs of 4.84 for insecticides (p = 0.008) and 1.53 for herbicides (p = 0.510) when these two variables were entered simultaneously. Table 2 lists adjusted ORs for pesticide-related exposures.

Table 2. Number of subjects/controls exposed, percentage subjects/controls exposed, crude odds ratios, and adjusted odds ratios for pesticide-related exposures

Variable	Subjects responding "yes"	Controls responding "yes"	Crude odds ratio	p	Adjusted odds ratio*	p
Herbicide exposure (>10 times/year)	18 (30.5)	6 (10.7)	3.46	0.011	3.22	0.033
Insecticide exposure (>10 times/year)	24 (42.9)	8 (15.1)	4.04	0.002	5.75	0.001
"Ever lived within ¼ mile of agricultural spraying?"	24 (48.0)	15 (31.4)	1.99	0.106	1.99	0.099
"Ever lived in a house that was fumigated?"	9 (15.0)	3 (4.6)	3.29	0.068	5.25	0.045

* Adjusted for age, age at diagnosis, race, sex, educational level, and family history of PD

Patterns of food, beverage, and cigarette consumption. Because some foods contain chemicals with neurotoxic potential, subjects/controls were asked about their past intake of several foods and beverages; cigarette use was also included in this category. Intake of nuts and seeds (estimated number of servings/week) 10 years before diagnosis (adjusted OR = 1.49, $p = 0.021$) was significantly associated with PD risk. PD was inversely associated with cigarette smoking (number of packs/day) 5 (adjusted OR = 0.50, $p = 0.027$), 10 (adjusted OR = 0.43, $p = 0.012$), and 15 years before diagnosis (adjusted OR = 0.37, $p = 0.005$). There were no significant differences ($p < 0.05$) in number of servings between subjects and controls at any of the four time periods for the following foods and beverages: milk, coffee, black tea, alcoholic beverages, fish, shellfish, blue cheese, arrowroot, and rare meat.

Models of independent predictors of PD. In the final phase of the analysis, three models of disease predictors were formulated to identify independent predictors of PD. The purpose of model 1 was to evaluate the effects of demographic and family history variables on PD risk; the variables of race (dichotomized as white/nonwhite), educational level (dichotomized as high school graduate or less/education beyond high school), sex, age, age at diagnosis, and family history of PD were included in this model. The total model was statistically significant and resulted in a 22.33 improvement in chi-square fit compared with the intercept only (chi-square intercept = 181.41, chi-square model 1 = 159.08, difference = 22.33, $p < 0.001$); two of the individual predictors, race (OR = 10.29, $p = 0.033$) and educational level (OR = 2.44, $p = 0.046$), were also significant. This model successfully predicted the disease status of 67.2% of subjects/controls.

The purpose of models 2 and 3 was to assess the extent to which model 1 could be improved by the addition of environmental variables into the equation. For model 2, the variables of exposure to insecticides and cigarette smoking 15 years prior to diagnosis were added to those variables from model 1; the model was highly significant and resulted in a significant improvement of chi-square fit beyond model 1, which contained only demographic and family history predictors (chi-square model 2 = 135.87, difference from model 1 = 23.21, $p < 0.001$). Both of the added predictors (exposure to insecticides, OR = 7.24, $p < 0.001$; cigarette smoking, OR = 0.32, $p = 0.002$) were also significant. The variable for herbicide exposure was not significant once insecticide exposure had been entered into the model because of multicolinearity between these two variables ($r = 0.512$, $p < 0.001$). Model 2 successfully predicted the disease status of 76.3% of subjects/controls.

After adding variables representing insecticide exposure and cigarette smoking, the predictive power of model 2 could not be improved by the addition of any variable. Because the inverse association between smoking and PD has been noted in numerous studies, this variable was removed from the equation to focus on other environmental predictors of PD risk. Model 3 included, in addition to the demographic and family history variables, the variables of exposure to insecticides, nuts and seed eating, residence in a town of less than 10,000 at time of diagnosis, and past residence in a house that was fumigated. Model 3 also resulted in a significant improvement of chi-square fit above model 1 (chi-square model 3 = 135.00, difference from model 1 = 24.08, $p < 0.001$). Two of the added predictors were statistically significant—exposure to insecticides (OR = 4.30, $p = 0.014$) and nuts and seed eating 10 years before diagnosis (OR = 1.50, $p = 0.035$); the other two predictors, residence in a town of 10,000 or less at time of diagnosis (OR = 2.35, $p = 0.090$) and past residence in a house that was fumigated (OR = 4.57, $p = 0.119$) were not statistically significant but improved the fit of the overall model. Model 3 successfully predicted the disease status of 72.5% of the subjects/controls. Tables 3, 4, and 5 summarize the three logistic regression models. Table 6 provides a comparison of the predictive power of the three models.

Table 3. Model 1: Logistic regression model for demographic and family-history predictors of PD

Individual predictors	Chi-square	p	Odds ratio	95% confidence interval Upper	Lower
Race	4.57	0.033	10.29	1.21	87.17
Educational level	3.97	0.046	2.44	1.01	5.87
Gender	3.18	0.075	0.48	0.22	1.08
Family history of PD	2.76	0.097	2.37	0.86	6.54
Age	2.53	0.112	0.95	0.90	1.01
Age at diagnosis	0.11	0.484	1.03	0.95	1.11

Total model	Chi-square	df	p
Intercept only	181.41		
Model (intercept and 8 variables)	159.08		
Difference	22.33	8	0.001

Discussion. The present study is the second investigation[21,24] to use a multivariate approach to examine the relationships between PD and water source, rural residency, pesticide exposure, and participation in farming. Findings from both of these studies point to a significant relationship between certain types of pesticide exposures and PD; the strength of this association remains significant when evaluated in the context of demographic and additional environmental variables. Significant ORs in the present study included exposure to insecticides (more than 10 times/year in any year) and exposure to herbicides (more than 10 times/year in any year). ORs between PD and "ever lived within ¼ mile of agricultural spraying?" and "ever lived in a house that was fumigated (tent placed over the

Table 4. Model 2: Logistic regression model containing variables from model 1 plus exposure to insecticides and cigarette smoking

Individual predictors	Chi-square	p	Odds ratio	95% confidence interval Upper	Lower
Race	7.80	0.005	27.84	2.70	287.17
Educational level	0.14	0.709	1.21	0.44	3.33
Gender	5.36	0.021	0.35	0.14	0.85
Family history of PD	2.21	0.137	2.39	0.76	7.53
Age	0.90	0.342	0.97	0.90	1.04
Age at diagnosis	1.45	0.228	1.06	0.97	1.15
Exposure to insecticides (> 10 times a year in any one year)	11.33	0.001	7.24	2.29	22.92
Cigarette smoking (packs/day 15 years before diagnosis)	9.26	0.002	0.32	0.15	0.67

Total model

	Chi-square	df	p
Intercept only	181.41		
Model (intercept and 8 variables)	135.87		
Difference	45.54	8	0.001

Table 5. Model 3: Logistic regression model containing variables from model 1 plus exposure to insecticides, nuts and seed eating, rural residency at dingnosis, and past residence in a house that was fumigated

Individual predictors	Chi-square	p	Odds ratio	95% confidence interval Upper	Lower
Race	7.16	0.007	26.03	2.39	283.09
Educational level	1.34	0.247	1.79	0.67	4.76
Gender	4.81	0.028	0.36	0.15	0.90
Family history of PD	1.01	0.315	1.81	0.57	5.76
Age	3.07	0.080	0.94	0.87	1.01
Age at diagnosis	2.34	0.126	1.08	0.98	1.18
Exposure to insecticides (> 10 times a year in any one year)	6.09	0.014	4.30	1.35	13.67
Nuts and seed eating 10 years before diagnosis	4.44	0.035	1.50	1.03	2.18
Residence in a town of 10,000 or less at time of diagnosis	2.87	0.090	2.35	0.87	6.34
Past residence in a house that was fumigated	2.43	0.119	4.57	0.68	30.84

Total model

	Chi-square	df	p
Intercept only	181.41		
Model (intercept and 10 variables)	135.00		
Difference	46.41	10	0.001

Table 6. Summary of logistic regression models

Model	Chi-square	df	Improvement in chi-square fit	df	p
Intercept only	181.41	131			
Model 1. Six demographic and family-history variables	159.08	125	22.33	6	0.001
			Improvement in chi-square fit from model 1		
Model 2. Variables from model 1 plus insecticide exposure and cigarette smoking 15 years before diagnosis	135.87	123	23.21	2	0.001
Model 3. Variables from model 1 plus insecticide exposure, nut and seed eating, past residence in a fumigated home, and residence in a town of 10,000 or less at time of diagnosis	135.00	121	24.08	4	0.001

home and insecticides applied)?" were elevated but not statistically significant. After adjusting for demographic and family history variables, the exposure of PD subjects to insecticides was five times that of controls ($p < 0.001$). Although logistic regression modeling revealed that insecticide and herbicide exposure were highly correlated, insecticide exposure was only moderately correlated with past residence in a fumigated house and with residence in a town of 10,000 or less. The correlation between insecticide exposure and fumigation was 0.189 ($p = 0.037$); that between insecticide exposure and rural residency was 0.219 ($p = 0.012$).

To date, the work of Barbeau et al[2] has provided the most compelling and methodologically sound evidence of an ecologic association between pesticide use and PD risk. Using several indices of PD prevalence and mortality, the authors found a correlation of 0.967 between region-specific PD prevalence in Quebec, Canada, and pesticide utilization per region (kg/yr, 1982). The recent population-based study by Semchuk et al[21] found an OR of 3.06 for herbicide exposure. However, two other studies by Koller et al[4] and Tanner et al[15] found no such association. However, as patients become more familiar with pesticide/PD hypotheses, there is an increased risk of selective recall in studies that rely on self-report data to estimate exposures. Patients sensitized to this information are more likely to have reflected over their past exposures to pesticides, and their responses to questions on this topic can result in inflated ORs for pesticide-related exposures. This scenario is an alternate explanation for the findings of the present study.

Lack of knowledge about agent-specific chemical risks and individual susceptibility makes it difficult to elucidate the nature of PD risk from pesticide exposure. The present study provides evidence on behalf of an association between pesticide exposure, most prominently to insecticide products, and PD.

"Rural living" themes predominate in the PD

epidemiology literature, and the highly intercorrelated variables of rural residency, drinking water source from a private well, participation in farming, and exposure to pesticides generally fall within this category. Rural residency has been operationalized different ways and has been interpreted as diversely as "living in a town of 169 or less"[7] to the present study, which used 10,000 as the population cutoff. Although the evidence that rural residents are overrepresented in the PD population is generally accepted, the meaning of this widely observed association is unknown. In the present study, rural residency at birth was inversely associated with PD; this was also the case with "ever lived or worked on a farm?" (adjusted OR = 0.38, p = 0.018). However, rural residency at time of diagnosis was significantly and positively associated with PD. The basis for these findings may lie in the manner in which farming and rural living were operationally defined. Operationalizing farming as a categorical variable (yes, no, don't know) may have oversimplified the adequate investigation of this exposure. Farming practices, and the chemical exposures they encompass, are extremely diverse and depend on the crop(s) produced, the types of pest controls utilized, and the proximity of land under cultivation to residential land. Farming may also be associated with increased exposure to chemicals indigenous to certain plants. The findings may also obscure a critical interval (ie, early adulthood) during which individuals are particularly susceptible to the untoward effects of chemical exposures.

Water source from a private or hand-dug well has also been hypothesized to play some role in the etiology of PD. Like the concept of rural residency, water source represents a highly variable and heterogeneous exposure. Because private wells are generally more shallow than those used for larger municipal supplies, the risk for PD is assumed to be due to some form of contamination (eg, pesticides, volatile organic compounds, minerals, trace elements) from the surface. In a study of PD clustering in Israel, Goldsmith et al[15] linked three kibbutzim with high PD prevalence to water source from a common aquifer. Recently, several authors[9,34] have attempted to find a dose-response relationship between PD risk and years of well water use; to date none has been observed. The present study found no significant differences in water source between subjects and controls at each of four time points.

Although some studies have attempted to find a dose-response relationship between rural exposures and PD (eg, number of years of rural living), a true risk factor is unidentified, making quantification of dose impossible. There is a great need for analytic epidemiology studies that measure a number of rural characteristics and use multivariate analysis techniques to separate out the relative contributions of each of the related variables. The challenge of untangling the associations between rural living and PD will lie, at least partly, in determining the constellation of environmental exposures for which rural residency is a proxy as well as in estimating the induction period of PD.

Two interesting and somewhat unexpected findings in the present study relate to the inverse association between DMSO and PD and to the positive association between PD and number of servings of nuts and seeds eaten per week. The strong association between the controls and DMSO is attributed to the selection of RA patients as the control group. DMSO, which was espoused in Oregon and elsewhere as a treatment for both rheumatoid and osteoarthritis, was used by many patients who applied the solvent topically to areas of pain and inflammation. Although subjects/controls were asked to address only those exposures that occurred before their diagnoses, the insidious onset of PD and RA demonstrates the difficulty in obtaining valid data on exposures that are closely wed to self-treatment of a disorder.

Questions about nut and seed consumption were included in part because of prior experience with a family in which three of four family members (mother, father, and 37-year-old son) were diagnosed with PD between the years 1961 and 1964.[12] The second son, who was not affected, was the only member of the family who did not subscribe to the family's unusual dietary regime which included large amounts of sunflower seeds. In the present study, the adjusted OR for nuts and seed eating 10 years before diagnosis was 1.49 (p = 0.021); the OR for 5 years before diagnosis was 1.34 (p = 0.055). This association is tempered by the known difficulties in obtaining accurate dietary recall data from patients for epidemiologic studies. However, because this association has now been seen twice in the literature, it might be worthwhile building this content area into future exposure history questionnaires.

The findings of an inverse association between cigarette smoking and PD risk are among the strongest found in the PD literature. First noted in 1966 by Kahn,[36] who observed a 0.23 mortality ratio between PD and cancer of the lung or bronchus, a significant inverse relationship between smoking and PD has since been noted in nine of 18 studies of PD.[5,22,29] Adjusted ORs for the present study ranged from 0.37 for 15 years before diagnosis (p = 0.005) to 0.50 for 5 years before diagnosis (p = 0.027). Reshef et al[28] found evidence of a dose-response relationship between smoking and drug-induced parkinsonism; however, most authors have argued against the possibility that smoking is biologically protective for PD. This conclusion has been supported by Golbe et al,[37] who found no association between any measure of PD severity and smoking duration or frequency in a survey of 3,600 PD subjects. Current thinking posits that smoking is a proxy variable for a personality type at risk. This idea proposes that persons regarded as neat, fastidious, and somewhat

rigid have a natural aversion to smoking; the rigid personality type is regarded as the true risk for PD, with nonsmoking representing a proxy variable for the at-risk personality.

Twenty-five percent of PD subjects had at least one relative with PD. That over 10% of controls also had a positive family history is not surprising; Golbe[31] noted that with a lifetime incidence of 2.5%, chance alone may account for a positive family history in as many as 15% to 20% of patients. Although the difference in the present study was significant (25% for subjects, 10% for controls), the questionnaire did not elicit the number of relatives at risk for PD, so we cannot rule out the possibility that this finding was due to differences in the numbers of relatives at risk rather than an increased disease prevalence in PD versus control family members. A related finding points to an unusually high frequency of ALS in both the subject (3.2%) and control groups (4.4%); this finding may be due to the way ALS was operationally defined in the study (a progressive weakness and loss of muscle function). In retrospect, this definition was not specific enough and could easily allow the misclassification of a number of neurologic and other disorders as ALS.

Because the goal in the present study was to maximize the opportunity to identify environmental risks for PD, the variable of family history of PD was held constant in computing the adjusted ORs. In the logistic regression analyses, family history of PD was often nonsignificant when evaluated in the context of environmental variables. For example, the adjusted OR for family history alone was 2.37 ($p = 0.097$); however, when variables representing exposure to insecticides, nuts and seeds, residence in a fumigated house, and rural residency were added to the equation, the OR for family history became 1.81 ($p = 0.315$). This finding emphasizes the relative importance of environmental variables, compared with family history of PD, in the present study.

One of the trade-offs in selecting a group of young-onset patients is the limited sample size that accompanies such a decision. The present study was severely restricted in its ability to interpret data on those exposures that occurred with low frequency in both the subject and control groups. Because of the small sample size, it was not possible to determine if negative findings on rare or unusual exposures (ie, head injuries, fungicides) occurred because there was no association between the exposure and disease outcome or because the study had inadequate power to detect an association. For example, Stern et al[28] found an OR of 3.0 for prior head injury in a study that examined risks in young-onset patients; in the present study the crude OR was elevated, but was not statistically significant (crude OR = 1.72, $p = 0.174$). With the current sample size (63 subjects, 68 controls) and an exposure rate of 4% in the control population, the exposure rate in the subject population would have to be 20% to have an 80% chance of detecting statistical significance ($\alpha = 0.05$). The recruitment of additional controls could have substantially increased the statistical power of the study. An additional limitation of the study relates to the possibility that subjects/controls misinterpreted their exposure status on those exposures that they might not have understood (eg, arrowroot ingestion, exposure to formaldehyde). The inclusion of a "don't know" option for all questions was intended to minimize this source of error. Finally, it is important to consider the statistical problems that occur with multiple comparisons; over 100 comparisons were made in the bivariate phase of the analysis. In such instances the investigator capitalizes on chance, leading to an increased probability of type I error. This is certainly a possibility with the present study, although the coherence of findings generally supports the notion that significant findings were not due to chance alone.

Both laboratory and applied research point to a multifactorial etiology in idiopathic PD; at the present time, the relative contribution of environmental agents to the expression of disease appears to be fairly large. The challenge lies in the identification of specific, rather than global, antecedents of disease. Findings from the present study add to the increasing weight of evidence that relates PD risk to exposure to pesticide-related products, mainly those serving as insecticides or herbicides. These research efforts demonstrate the need for additional field studies that have the potential to identify specific pesticide products as well as other candidate triggers of disease.

Acknowledgments

The authors wish to thank Roderick Hooker, PA, and Phillip Ballard, MD, for their assistance with patient referrals and Jonathan Fields for statistical support.

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Risk factors for Parkinson's disease

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Article abstract—Parkinson's disease (PD) has been associated with rural living, well-water consumption, and pesticide exposure; however, the individual risk contribution of these variables has not been established. We examined social and medical histories of predominantly rural populations to determine relative risk factors for PD. Patients and controls were surveyed regarding residency, occupation, medical history, and social and dietary habits. An initial multiple logistic regression model was confounded by excessive variable collinearity. Principal factor analysis yielded three factors: rural living (including years of rural residency and ground-water use), pesticide use, and male lifestyle (male gender, head trauma, male-dominated occupations). Other variables did not load in factor analysis and were entered separately, with the three factor scores, in a second multiple logistic regression model. Significant predictors of PD emerged (in order of strength): pesticide use, family history of neurologic disease, and history of depression. The predicted probability of PD was 92.3% (odds ratio = 12.0) with all three predictors positive. Pesticide use (distinguishable from rural living) can be considered a risk factor for the development of PD, with family history of neurologic disease and history of depression serving as weaker predictors of PD.

NEUROLOGY 1993;43:1693-1697

While the etiology of Parkinson's disease (PD) is unknown, increasing evidence supports the hypothesis that environmental factors contribute to its cause.[1] The occurrence of PD has been linked to rural residency and associated lifestyle features, including farming and well-water use.[2-7] In studies finding an association between rural life and PD, it can be inherently difficult to examine specific aspects of rural life for causation. If a control study population is significantly more urban when compared with a group of PD patients, features common to rural life, such as well-water consumption and farming, would be expected to be more common among the patients, but the significance of such associations is obscured. To distinguish and refine the known risk factors for PD, we examined lifelong sociodemographic features of PD patients and unaffected individuals with a high incidence of rural backgrounds.

Methods. The study was conducted at a rural site and an urban site. Hays, Kansas, served as the rural site. Located in the wheat-producing western half of the state, Hays has a population of 17,000. At the rural study site, PD subjects (n = 31) were recruited via a PD outreach clinic in Hays, and controls (n = 45) were recruited via news media releases and from a senior citizens' luncheon program. The urban site was Kansas City, Kansas. The metropolitan Kansas City area had a 1990 population of 1.6 million. At the urban study site, PD subjects (n = 32) were recruited from a university-based PD clinic and from PD support groups in the urban area. Urban study site controls (n = 31) were recruited from a university hospital neurology clinic; also, PD subjects were asked to recruit nonfamily controls, accounting for 10 of the urban study site control subjects.

All subjects were examined by a neurologist (J.P.H.). The diagnosis of PD was based on the presence of two or more of the cardinal signs of PD (tremor, rigidity, and bradykinesia) and responsiveness to levodopa. Patients with historical features or signs and symptoms suggesting atypical or secondary parkinsonism were excluded. Controls presenting with any parkinsonian signs or evidence of other neurodegenerative diseases (eg, Alzheimer's disease, essential tremor) were excluded.

Information was collected by means of a self-administered questionnaire returned by mail, with an average return time of 8 days (range, 2 to 18). The questionnaire was modified from an instrument previously employed.[6] Included were categoric responses to queries determining whether subjects had ever lived or worked on a farm; used pesticides for more than 20 days during any 1 year; used pesticides for more than 20 days a year for more than 5 years; lived or worked on a farm with livestock; vaccinated animals; smoked more than 100 cigarettes lifelong; suffered from an infection involving the nervous system; sustained a head trauma for which medical attention was sought or which rendered the subject unconscious; received professional help (from a doctor or counselor) for depression; had social, medical, personal, or financial problems due to alcohol use; and consumed fresh produce more than twice weekly. Subjects provided

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Presented at the 44th annual meeting of the American Academy of Neurology, San Diego, CA, May 1992.

Received July 17, 1992. Accepted for publication in final form February 1, 1993.

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Table 1. Demographic data

	Urban study site		Rural study site	
	PD cases	Controls	PD cases	Controls
Current residence*				
Rural	8 (25%)	4 (13%)	21 (68%)	11 (25%)
Urban	24 (75%)	27 (87%)	10 (32%)	33 (75%)
Gender*				
Male	17 (53%)	14 (45%)	17 (55%)	20 (46%)
Female	15 (47%)	17 (55%)	14 (45%)	24 (55%)
Mean age (yr ± SD)	69.3 ± 8.5	67.6 ± 11.0	69.0 ± 10.2	69.8 ± 9.2

* Expressed as number of subjects (% total subjects)

a listing of their country of birth and those of their parents and grandparents, and were also asked to list neurologic illnesses (descriptors of such conditions were provided) among their grandparents, parents, siblings, and children. Subjects were asked to characterize their ethnicity based on a multiple-choice listing. Lifetime smoking history and estimation of alcohol consumption was obtained. Lifelong occupational histories were obtained. The questionnaire specifically listed occupations known to be most common in the Hays, Kansas, region based on data obtained from a local civic organization. In addition, subjects were asked to supply descriptions of employment, if not specifically listed, to account for all work experiences (full- or part-time) lifelong. Residencies were obtained as continuous data. For each residence, subjects were asked to specify the water source as city supplied, cistern, spring, or private well. For analysis, continuous data were collapsed into categories. Rural living was defined as residency in a town of less than 2,500 population; population statistics were obtained from the US Census Bureau for each residence at the time the subject lived there. Ground water was defined as water from a private well or spring. Years of rural residencies, groundwater use, and farming were analyzed as follows: <20 versus 20+ years total lifelong, <25 versus 25+ years total lifelong, <30 versus 30+ years total lifelong, <20 versus 20+ years total when <40 years old, <25 versus 25+ years total when <40 years old, <30 versus 30+ years total when <40 years old. The stratification based on variable exposures during early decades of life (<40 years old) was conducted because of earlier work suggesting early-life rural exposures may be more strongly linked to PD.[5] The reliability of questionnaire responses was checked by phone follow-up with 10% of all subjects.

Statistical analysis was performed on the SAS statistical software package using chi-square tests and multiple logistic regression, with alpha set at 0.05. Initial logistic regression modeling was confounded by excessive variable collinearity and, therefore, variables were entered into principal factor analysis with promax oblique rotation prior to final analysis. Promax oblique rotation method was chosen in order to obtain what was anticipated to be the truest configuration of the factors, given the excessive collinearity observed in the initial logistic regression model. Promax rotation allows for intercorrelation of factors if such exists.

Results. Demographic features of the study populations are described in table 1. The study was completed by all recruited PD subjects; one recruited control subject failed to complete the questionnnire. Three PD subjects at the urban site and five at the rural site had assistance (six assistants were spouses and two were adult children). No control subject had assistance. No errors or omissions in responses were detected in phone follow-up interviews.

Table 2. Principal factor analysis*

	Rural living factor	Pesticide factor	Male lifestyle factor
Eigenvalue	6.05	1.97	1.16
	Loadings	Loadings	Loadings
Age <40 years			
>20 yr rural living	0.918		
>20 yr ground-water use	0.908		
>20 yr farming	0.905		
Life-long			
>20 yr farming	0.905		
>20 yr ground-water use	0.898		
>20 yr rural living	0.892		
Ever work or live on farm	0.669		
Ever vaccinate animals	0.365		
Pesticide/herbicide use			
>20 d/yr for >5 yr		0.921	
>20 d/yr		0.889	
Gender			−0.697†
Ever smoke cigarettes			0.601
Alcohol abuse			0.568
Agribusiness occupation			0.494
Shared variance explained (cumulative)	62.2%	82.4%	94.4%

* From principal factor analysis with promax oblique rotation, loadings <0.360 deleted. Loadings are given for individual variables within each factor.
† Negative loading = male.

The initial logistic regression model was constructed to attempt to determine the contribution of each study variable to the likelihood of occurrence of PD; however, the analysis was unsuitable for interpretation because several variables were highly correlated (excessive collinearity). To distinguish which variables formed correlated clusters and which could be used separately, all responses were submitted to a principal factor analysis. This process yielded three factors (table 2). The rural living factor included (in order of strength of association) having lived for more than 20 years in a rural area when less than age 40, ground-water use for more than 20 years when less than age 40, having farmed as an occupation for more than 20 years when less than age 40, having farmed as an occupation for more than 20 years lifelong, groundwater use for more than 20 years lifelong, having lived in a rural area more than 20 years lifelong, having ever lived or worked on a farm, and having ever vaccinated animals. The pesticide factor included having used pesticides for more than 20 days in any given year and having done so for more than 5 years. The male lifestyle factor included male gender, having ever smoked cigarettes, history of alcohol abuse (alcohol use interfering with employment or personal life), and history of occupations pertaining to agriculture (examples: farmer, grain elevator worker). In sum, these three

factors accounted for 94% of the shared variance among responses.

The occurrence of exposure to the chief variables of interest in subjects from the two study sites were compared. The rural living factor was the only variable differing significantly among the subjects based on site (table 3); positive exposure to the rural living factor was more common in subjects recruited at the rural site compared with the urban site when examining all subjects, cases-to-cases, or controls-to-controls, but did not serve to distinguish PD cases from controls. Data from the two sites were pooled prior to the final analyses. A second logistic regression model was then constructed, including the three factor scores (rural living, pesticide use, and male lifestyle) as new variables, with individual variables that did not load into the principal factor analysis. The nonloading variables included neurological family history, ethnicity, history of CNS infection, history of head trauma with loss of consciousness, history of depression, age under 65, and fresh produce consumption. From this analysis (both stepwise and nonstepwise), three significant predictors of PD emerged in order of strength: the pesticide factor, neurologic disease family history, and history of depression (table 4). When all three predictors were positive, the probability for PD in the subject was 92.3% (odds ratio = 12.0); when all three predictors were negative, the probability for PD in the subject was 28.7% (odds ratio = 0.4). Because depression may be viewed as a common clinical feature of PD, the analysis was repeated, deleting the history of depression variable. The pesticide factor and neurologic family history remained as significant predictors of PD (pesticide factor, $p = 0.005$, and neurologic family history, $p = 0.008$).

Table 3. Comparison of subjects from urban and rural sites

Variables	Urban site exposures n (%)	Rural site exposures n (%)
Age 65+ yr	19 (30)	23 (31)
Rural living factor	16 (25)*	46 (61)*
Pesticide factor	10 (16)	21 (28)
Male lifestyle factor	27 (43)	37 (49)
Neurologic family history	16 (25)	15 (20)

* Exposure to the rural living factor differed significantly in urban-site versus rural-site subjects (cases and controls combined), urban-site versus rural-site cases (31% versus 66% exposed), and urban-site versus rural-site controls (19% versus 53% exposed). Chi-square analysis ($df = 1$) for each comparison, respectively value = 15.358, $p = 0.0001$; value = 7.570, $p = 0.006$, value = 8.930, $p = 0.003$.

Discussion. The notion that the occurrence of PD is associated with rural living or farming dates to the mid-1980s.[2-4] In the intervening years, the issue has been examined numerous times, with most reports corroborating but some refuting the association between PD and rural living.[5-9] The discrepancies in results may be attributable, in part, to differing study populations and methodology. Clearly, rural living is not the sole determinant of PD because there are many patients who lack such a history. Assuming that rural living is a link to the cause of PD, distinguishing which feature of rural living serves as the actual risk factor for PD is difficult. Consideration has been given to well-water consumption and its constituents, including chemicals and minerals.[10] It is also possible that certain ethnic groups are overrepresented in some rural areas and that thus a genetic component could contribute to the association between rural living and PD. Aging itself is associated with rural living because one of four Americans over age 65 resides in a rural setting.[11]

To examine the relative contribution of rural living lifestyle features to the occurrence of PD, we studied populations in which rural residency was common. Most of our subjects (both patients and controls) had a history of living in sparsely populated, midwestern regions. Our most nonrural study group was the control population at the urban study site, yet even in this group, 51% had lived in a rural setting for more than 20 years. Rural living was so common among these subjects that it did not serve to distinguish controls from patients and

Table 4. Predictive variables for PD

	Nonstepwise logistic regression				Stepwise logistic regression*			
	χ^2	p Value	OR	95% CI	χ^2	p Value	OR	95% CI
Pesticide use	6.94	0.0084	3.15	1.30-6.63	8.24	0.0041	3.42	1.27-7.32
Neurologic family history	5.11	0.0238	2.84	1.12-7.21	7.04	0.0080	3.18	1.22-7.05
History of depression	2.95	0.0284	2.95	1.08-8.01	4.29	0.0383	2.74	1.07-7.57

χ^2 Approximate chi-square statistic to remove terms ($df = 1$).
OR Odds ratio.
CI Confidence interval
* Chi-square statistics for improvement to the prediction were significant at each step ($p = 0.015$, 0.010, 0.030, respectively). Hosmer-Lemeshow goodness-of-fit chi-square statistic = 0.240, $df = 5$, $p = 0.999$. C.C. Brown goodness-of-fit chi-square statistic = 0.261, $df = 2$, $p = 0.878$

it could not be successfully differentiated from other rural life features, including ground-water consumption and farming as an occupation. On the other hand, pesticide use emerged as a variable distinct from rural living and served as a strong predictor of PD among these subjects. In some reports, pesticide exposure has not been associated with PD.[6-12] This seeming incongruence may be due to the high degree of rural exposure in all our subjects, making it more likely that a specific feature of rural life, such as pesticide use, could be distinguished and analyzed as a risk factor. Our finding supports the hypothesis that the neuronal degeneration of PD is determined, in part, by environmental toxin exposure.[13] It is consonant with the results of Tanner et al,[14] who demonstrated PD to be less common among rural versus urban residents in China, where chemical weed and pest control has not been extensively employed. It is inconceivable, however, that PD is solely due to pesticides because not all patients have had such an exposure. In addition, PD as a clinical entity predates the development and widespread application of these chemicals. Isolated reports have been issued linking pesticide exposure to individual cases of parkinsonism in grain handlers, farmers, and workers in pesticide plants.[15-17] Semchuk et al[18] found occupational herbicide exposure to be the sole risk factor for PD when examining several agriculturally related factors in a case-control study of Calgary residents. Evidence suggesting a higher occurrence of PD in agricultural regions, as reported by Barbeau et al,[4] further supports the association of pesticide exposure and parkinsonism. When taken in consort with our own work, the most likely explanation for the role of pesticide exposure in PD is that it is but one of a number of precipitants of PD. Furthermore, the toxicity of such exogenous factors may be determined by inherent vulnerability within the individual. For example, prior nigrostriatal injury, aging, or genetic factors may determine individual susceptibility to such compounds.[19]

Second to pesticide use, the next most powerful predictor for PD in our subjects was a reported family history of neurologic disorders, including PD, Alzheimer's disease, tremor, dementia, or palsy. No attempt was made to differentiate these ailments because corroboration of diagnoses based on clinical examination or medical records was not possible. The stronger family history in our PD subjects may reflect recall bias because the patients may have increased awareness of PD and related disorders. Control subjects may be unfamiliar with such conditions and have little reason to ascertain and recall neurologic diagnoses in family members. Alternatively, this finding may be taken as evidence supporting the notion that PD and similar ailments are genetically determined. A familial tendency for PD has been postulated for decades,[20] but a clear inheritance pattern is rarely distinguishable.[21,22] The nature of the conjectured genetic defect also remains elusive; however, various metabolic defects in PD have been reported,[23-26] raising the possibility that PD is the result of genetic error that becomes clinically manifest with cumulative exposure to endogenous or exogenous toxins.[27]

Reported history of depression was the third strongest predictor for PD in this study. As with family history, reports of depression among PD patients may be biased compared with controls' responses. It is possible, for instance, that PD patients have more ready access to health care or would more easily recognize depressive symptomology and seek treatment. Patients may be more candid in reporting psychological problems because they are likely to be well motivated to respond completely and correctly to a survey that may shed light on the cause of their ailment. Despite these reservations, our findings can be taken as corroboration of the long-recognized association between depression and PD. Depression is reported to occur in 20 to 90% of PD patients, depending on the methodology and study population.[28-31] Depression has either not correlated or has been only weakly linked to motor impairment severity.[30,32,33] Thus, it is unlikely that depressive symptoms in PD patients are merely in response to the disabling nature of this illness, ie, "reactive" depression. In many patients, depression predates all motor signs and symptoms.[31-35] The nature of the relationship between depression and PD remains uncertain. Depression and PD may represent two totally distinct clinical entities frequently coexisting because of overlapping at-risk populations. Alternatively, depression can be viewed as an integral clinical feature of PD and, therefore, could not be appropriately analyzed as a risk factor for PD. With this last consideration in mind, we reconstructed our statistical model, deleting the history of depression variable; pesticide use and neurologic family history remained as significant predictors of PD among our subjects.

Several variables examined were not predictive of PD. For example, head trauma, ethnicity, history of CNS infection, and fresh-produce consumption were not found to be significantly more frequent among PD subjects compared with controls. These negative findings do not exclude such variables as possible risk factors for PD. The sample size was likely too small to examine adequately some of these variables, such as CNS infections. In addition, some variables could not be sufficiently distinguished from others. For example, men tended to have a history of head trauma, smoking, problem drinking behavior, and occupations related to agriculture; thus, these variables were ultimately analyzed as a single factor.

When studying risk factors for PD in individuals with high occurrence of rural exposure, we found that pesticide use; family history of neurologic disorders, and history of depression emerged as risk factors for PD. It is possible that these results are a reflection of selection bias, ie, inherent differences

in the selection of cases versus controls other than the presence or absence of PD; however, the lack of significant differences in age, sex, lifelong rural residency, and occupation suggests that selection bias was not an important influence. The demonstration of these risk factors further supports the hypothesis that the etiology of PD is related to both environmental and genetic factors. These findings should prompt speculation and investigation into the identity of specific chemical agents that may be culpable and also raise further questions as to the nature of a familial influence in PD.

Acknowledgments

The authors are grateful to the patients and staff of the Parkinson's disease clinics at the University of Kansas Medical Center (Kansas City, KS) and the Northwest Kansas Area Health Education Center (Hays, KS).

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NEURODEGENERATION, Vol. 2, pp 1-7 (1993)

Iron-melanin Complex is Toxic to Dopaminergic Neurons in a Nigrostriatal Co-culture

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In Parkinson's disease, biochemical and nuclear magnetic resonance analyses have shown a selective and highly significant elevation of iron in melaninized dopaminergic neurons of the substantia nigra. Lines of evidence indicate that an iron-melanin interaction could be crucial to degeneration of dopaminergic neurons in Parkinson's disease. In this paper, we report the cytotoxic effects of iron and melanin on dopaminergic neurons using a rat nigrostriatal co-culture. Immunuhistochemistry, using anti-tyrosine hydroxylase (TH) antibodies, reveal many TH-positive cells. Iron (Fe^{3+}) and dopamine-melanin were added to the culture wells at different concentrations with or without 150 μM deferoxamine mesylate. After incubation with Fe^{3+} alone, the number of TH-positive cells decreased in a dose-dependent manner. Moreover, the group incubated with 5 μM iron-melanin complex in co-culture resulted in 68% cell loss compared with the group incubated with 5 μM iron alone. There was no significant reduction in the number of TH-positive cells in cultures pretreated with deferoxamine mesylate. These results suggest a neurotoxic effect of iron-melanin complex on dopaminergic neurons *in vitro*. In addition, we demonstrated an induction of lipid peroxidation by the iron-melanin complex in our co-culture system, suggesting that the iron-melanin complex could be a chemical mediator of oxygen radical-induced neurodegeneration in melaninized dopaminergic neurons.

Key words: Iron, melanin, nigrostriatal co-culture, lipid peroxidation, Parkinson's disease

THE CAUSE of death of neurons of the substantia nigra (SN) in Parkinson's disease (PD) is still unknown. However, Ben-Shachar *et al.* (1991) suggested that an iron-melanin interaction could be crucial in the initiation of neurodegeneration resulting from oxidative stress. Recent studies have shown a selective and highly significant elevation in total iron, especially Fe^{3+}, to occur in the SN of patients with PD (Earle, 1968; Drayer *et al.*, 1986; Dexter *et al.*, 1987, 1991; Rutledge *et al.*, 1987; Reiderer *et al.*, 1989). Since iron facilitates formation of toxic hydroxyl radicals (·OH) and superoxide (O_2^-) anions as well as hydrogen peroxide, the amount of free radicals may be increased in the SN of patients with PD compared with normal, thereby playing a role in the cellular damage. In addition, several metals and drugs, especially MPP^+, can bind to neuromelanin and potentiate its toxicity on the dopaminergic neurons (Bruenger *et al.*, 1967; Larsson & Tjalve, 1979; D'Amato *et al.*, 1986; Ben-Shachar *et al.*, 1991). Therefore, interest has been focused on the role of iron-melanin complex in neurodegenerative disorders.

In-vitro studies using neuronal cultures are an important tool for investigating the mechanism of iron-mediated nigral cell loss in PD. Most previous studies have been done using mesencephalic cultures containing the SN. To better investigate any toxic effects of candidate substances, it seems more appropriate to use nigrostriatal co-cultures, because they simulate more closely the in-vivo condition with trophic factors from the target neurons. The purpose of this paper is to report toxic effects of iron-melanin complex on nigrostriatal co-cultures.

Materials and Methods

Materials

Chemicals used were of an analytical grade, all being purchased from Sigma, except for $Fe_2(SO_4)_3$ which was obtained

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Received 24 November 1992; accepted for publication 12 February 1993

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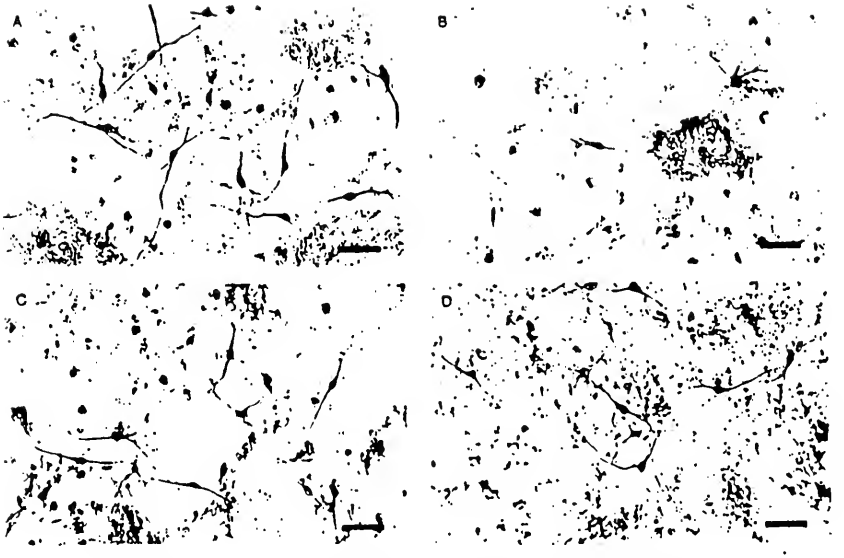

Figure 2. Photomicrographs of TH positive neurons of the nigrostriatal co-culture on day 7. The following substances were added on day 3: (A) vehicle; (B) Fe^{3+} (25 μM) + dopamine-melanin (3 μg/ml, respectively) complex; (C) desferrioxamine mesylate (150 μM) + Fe^{3+} (25 μM) + dopamine-melanin (3 μg/ml); (D) dopamine-melanin (3 μg) alone. Fe^{3+} was applied as Fe^{3+}-ADP complex. See text for detail. Scale bar = 50 μm.

increase in free radical formation may not be the only mechanism underlying iron toxicity.

In humans and monkeys, extensive loss of neurons from the SN has been reported following intoxication with low doses of MPTP, while in rodents, even at high doses, neuronal cell loss is mild. It has been postulated that species which have neuromelanin in their dopaminergic cells are more susceptible to MPTP toxicity (Burns *et al*., 1984). The presence of neuromelanin in primates, but not in rodents, suggests a role of neuromelanin in the vulnerability of primates to MPTP toxicity. Furthermore MPP$^+$ binds with relatively high affinity to neuromelanin (D'Amato *et al*., 1986) as do other drugs. This may account for the vulnerability of neuromelanin containing neurons to MPP$^+$ toxicity. Recently, Hirsch *et al*. (1988) demonstrated a greater vulnerability of the neuromelanin-containing dopaminergic neurons to the neurodegenerative process in PD. Furthermore, Ben-Shachar *et al* (1991) demonstrated a high specific capacity of melanin to bind iron. These latter authors suggested that an iron-melanin interaction could be crucial in initiating neurodegeneration following oxidative stress. A selective increase in lipid peroxidation in the SN of patients

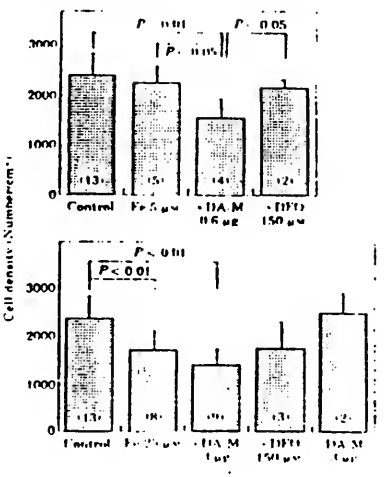

Figure 4 Effect of Fe^{3+} and dopamine melanin complex on the number of neurons positively labeled by TH immunohistochemistry in the nigrostriatal co-culture. (A) Control, vehicle; Fe^{3+} 5 μM; Fe^{3+} alone; + DA-M 0.6 μg/ml; 5 μM Fe^{3+} + DA-M 0.6 μg/ml; + DFO 150 μM; 5 μM Fe^{3+} + DA-M 0.6 μg/ml; + Fe-free deferoxamine mesylate. (B) Control, vehicle; Fe^{3+} 25 μM; 25 μM Fe^{3+} alone; + DA-M 3 μg/ml; 25 μM Fe^{3+}; + DA-M 3 μg/ml; + DFO 250 μM; 25 μM Fe^{3+} + DA-M 3 μg/ml; + Fe-free deferoxamine mesylate; DA-M 3 μg/ml; DA-M 3 μg/ml alone. The numbers in the parentheses indicate the number of wells.

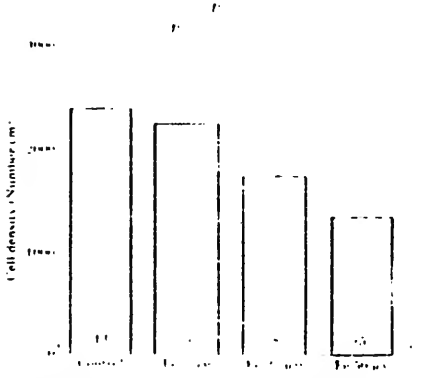

Figure 3 Effect of Fe^{3+} on the number of neurons positively labeled by TH immunohistochemistry in the nigrostriatal co-culture. The culture conditions are the same as described in the legend for Figure 2. The numbers following Fe^{3+} represent the concentration of Fe^{3+} added to the culture. The control was treated with the vehicle. The numbers in the parentheses indicate the number of wells.

with PD has also been reported (Dexter *et al*., 1989). Our present study has shown it necessary for neuromelanin and iron to co-exist before lipid peroxidation can take place. This may be an underlying explanation for the selective loss of melanin containing neurons in PD.

This is the first report showing detection of lipid peroxidation using a primary nigrostriatal co-culture. Destruction of dopaminergic neurons, as quantified by loss of TH positive neurons, correlated with an increase in lipid peroxidation. Moreover, incubation with the iron-melanin complex in the nigrostriatal cultures resulted in a marked dopaminergic cell loss and an increased production of MDA; interestingly, melanin itself had no cytotoxic effects on

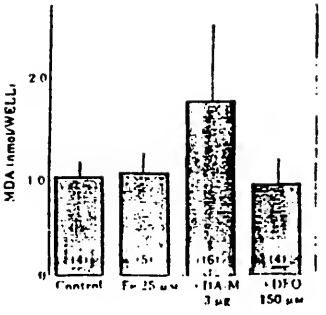

Figure 5 Lipid peroxidation by iron and dopamine melanin. Lipid peroxidation was measured as the amount of malondialdehyde (MDA) formation in the nigrostriatal co-culture. Control: basal lipid peroxidation, Fe^{2+} 25 μM, 25 μM Fe^{2+} + DA-M 3 μg/ml, 25 μM Fe^{2+} + DA-M 3 μg/ml + DFO (150 μM, 2 h) + 150 μM deferoxamine mesylate. Two wells were combined to make one sample. Values are expressed as nanomoles of malondialdehyde (MDA) per sample. The numbers in the parentheses indicate the number of samples assayed.

cause underlying degeneration of the SN. Free iron may induce DNA mutations by the generation of oxygen free radicals (Loeb *et al.*, 1988).

In conclusion, our present studies indicate that while iron is neurotoxic to cultured neurons, in a dose-dependent manner, the iron-melanin complex has a more pronounced neurotoxic effect.

Our co-culture system, simulating more closely the in-vivo nigrostriatal system, compared with conventional mesencephalic cultures alone, appears to be a good model for studying the toxic effects of substances which could potentially be responsible for death of cells in the SN in PD.

Acknowledgements

This work was supported by the Grant-in-Aid for Encouragement of Young Scientists and in part by the Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science and Cultures. We would like to thank Ms Kumiko Nefuji and Ms Hiroyo Yoshino for their technical assistance.

cultured dopaminergic neurons. Regarding the source of MDA, we believe that there is a considerable contribution from the background non-dopaminergic cells. However, effects of iron will be more deleterious in melanonized cells because melanin may potentiate the formation of activated oxygen species as neuromelanin catalyses the reduction of Fe^{3+} to Fe^{2+}. The production of OH and lipid peroxidation in the presence of melanin is significantly greater when Fe^{2+} is present (Youdim *et al.*, 1989) because H_2O_2 and melanin may serve as catalysts for the conversion of Fe^{3+} to Fe^{2+} (Halliwell *et al.*, 1986). However, incubation with iron alone had no effect on the amount of MDA, although it caused a reduction in the number of dopaminergic neurons, as revealed by immunohistochemistry. Therefore factors other than lipid peroxidation alone seem to play a role in iron-mediated dopaminergic cell loss. As we have demonstrated in PD, a partial decrease in the amount of mitochondrial Complex I subunits (Mizuno *et al.*, 1989), an accumulation of deleted mtDNA (Ikebe *et al.*, 1990) and point mutations of mtDNA (Ikebe *et al.*, 1992), interaction of iron with DNA may be another possible

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from Wako. The TH (tyrosine hydroxylase) antiserum, raised against purified bovine adrenal tyrosine hydroxylase, was purchased from Eugene Tech International, Inc.

Cell culture

Cultures were prepared from the ventral mesencephalon and neostriatum of rat embryos at embryonic day 14 (E14) as previously described (Nishi *et al.*, 1990) with some modifications. The neostriatum and ventral mesencephalon were dissected in cold calcium- and magnesium-free Hank's balanced salt solution (CMF HBSS). These tissues were mechanically dissociated and plated into 24-well culture dishes pretreated with polyethylenimine. Culture chambers were prepared by placing sterile fluoropolymer plastic film (Aclar®) on the bottom of chambers. The cell density in each well was 2.4×10^5/cm^2. The ratio of the cellular population between the ventral mesencephalon and the neostriatum was 1:2.

Cultures were maintained for the first 4 days in MEM (Modified Eagle's Medium)/F12 supplemented with insulin (10 μg/ml), streptomycin (50 μg/ml), penicillin (50 U/ml), and 10% fetal bovine serum. Thereafter the cells were treated with MEM/F12 with 5% newborn calf serum/5% horse serum, insulin, streptomycin/penicillin and 0.5 μM cytosine arabinoside to control glial proliferation.

Immunohistochemistry

Cultures grown on coverslips were fixed with 4% paraformaldehyde in 0.01 M PBS. They were then rinsed in PBS and incubated with 0.25% Triton X-100 for 15 min. After rinsing in PBS, cultures were incubated with 1% hydrogen peroxide for 30 min. They were then rinsed in PBS and incubated in 5% normal goat serum for 20 min. After rinsing in PBS they were incubated with the tyrosine hydroxylase antiserum (TH dilution 1:800 in 0.1% Triton X-100 and 1% BSA in PBS) for 48 h. Cultures were then rinsed in PBS, incubated for 1 h with biotinylated anti-rabbit IgG (Vector Labs, dilution 1:200 in PBS), rinsed and incubated with avidin-biotin-conjugated HRP for 1 h (ABC Vector, dilution 1:100 in PBS). After rinsing, the HRP-antibody complex was visualized with diaminobenzidine. Coverslips were then rinsed in PBS and mounted with glycerin onto glass slides.

Preparation of dopamine-melanin

The synthetic melanin (dopamine-melanin) was prepared by auto-oxidation of 3,4-dihydroxyphenylethylamine (dopamine) according to the method described by Ito *et al.* (1976), with modifications by D'Amato *et al.* (1986) and by Ben-Shachar *et al.* (1991).

A solution of 2 mM of dopamine in the presence of 100 μM copper sulfate was prepared in 200 ml of 0.05 M Tris-HCl buffer, pH 8.0. The mixture was stirred in a beaker for 24 h at room temperature (25–26°C). Oxidation was stopped by the addition of concentrated HCl to pH 2. The pellet of melanin was obtained by centrifugation at 2800 *g* and washed three times with 3 ml of 0.01% KCl solution. The final pellet was homogenized in 10 ml water with a glass-teflon homogenizer and transferred quantitatively into a dialysis bag. The homogenized pellet of melanin was dialysed against distilled water for 48 h. Then the melanin suspension was lyophilized and stored at 4°C. The lyophilized melanin was resuspended in distilled water and sonicated (100 μA) for 3 min prior to use.

Fe^{3+} and melanin administration

Cultured tissues were incubated with a medium containing 5, 25 and 50 μM Fe^{3+} in the form of Fe^{3+}-ADP complex prepared according to the method of Sugioka & Nakano (1982) with or without synthetic melanin for 48 h. Control tissues were incubated with the vehicle and the culture medium without Fe^{3+} for the same period. Morphological changes caused by Fe^{3+} and melanin administration were analysed by immunocytochemistry. The ratio of Fe^{3+} to dopamine-melanin was set at 100 μM of Fe^{3+} to 12 μg/ml of dopamine-melanin according to the report of Ben-Shachar *et al.* (1991) to yield maximum lipid peroxidation. Furthermore, we examined the effect of pretreatment by deferroxamine, a potent chelator of Fe^{3+}, to confirm the active involvement of iron.

Measurement of lipid peroxidation in co-cultures

Lipid peroxidation was measured using co-cultures on day 7. The co-cultures were divided into four groups. The first group represented the control. The second group was treated with 25 μM of iron. The third group with 25 μM of iron-melanin complex and the fourth group with 1 mM deferroxamine mesylate prior to the addition of the iron-melanin complex. The control group was added the vehicle alone. These cultures were incubated at 37°C for 60 min. To each culture dish, 0.5 ml of 0.1% sodium dodecyl sulfate was added, and cultured cells were scraped and transferred to a test tube. To this solution, 4.0 ml of $N/12$ H_2SO_4 was added and mixed. Then, 0.5 ml of 10% phosphotungstic acid was added and mixed well. After standing at room temperature for 5 min, the mixture was centrifuged at 2000 *g* for 10 min. The supernatant was discarded and the sediment mixed with 2.0 ml of $N/12$ H_2SO_4 and 0.5 ml of 10% phosphotungstic acid. This mixture was centrifuged at 2000 *g* for 10 min. The pellet was suspended in 4.0 ml of distilled water, then 1.0 ml of thiobarbituric acid (TBA) reagent was added. The reaction mixture was heated at 95°C for 60 min in a boiling water bath. After cooling with tap water, 5.0 ml of n-butanol was added and the mixture shaken vigorously. After centrifugation at 2000 *g* for 15 min, the n-butanol layer was retained for fluorometric assay with excitation wave length at 515 nm and emission wave length at 553 nm. The equipment used was a fluorescence spectrophotometer (Hitachi 850). The lipoperoxide concentration of pellet was calculated according to the following equation: Lp = $0.5 \times F/f$ (nmol/well) where 'f' indicates the fluorescence intensity of the sample and 'F' the fluorescence intensity of the standard solution which contained 0.5 nmol 1,1,3,3-tetraethoxypropane. The Lp was expressed as the amount of malondialdehyde (MDA) formed (Yagi, 1976).

We confirmed (Figure 1) a linear relationship between the amount of the tissue used and the fluorescence intensity.

Results

Toxic effects of Fe^{3+} on dissociated cells in co-culture

After incubation with Fe^{3+} (5 μM or 25 μM) and dopamine melanin, the number of TH-positive cells decreased markedly without any observable effect on the 'background' population of the TH negative cells when visualized by phase contrast microscopy. There was no significant decrease in the number of TH positive cells in the culture pretreated with deferoxamine mesylate (Figure 2). However, incubation with more than 50 μM Fe^{3+} damaged all cells which peeled from the culture dish. The number of TH-positive cells was counted in a 1 cm^2 area of each culture dish. Figure 3 shows the effect of various concentrations of the Fe^{3+}-ADP complex without dopamine melanin on the TH positive cell density. Cell density did not decrease significantly with 5 μM Fe^{3+} but then decreased significantly as the amount of iron increased. Furthermore, the number of TH positive cells was markedly decreased by the addition of dopamine-melanin complex to the iron-ADP complex, and this decrease was prevented by deferoxamine mesylate (Figure 4). Dopamine melanin itself had no cytotoxic effects.

Measurement of lipid peroxidation in co-culture

Figure 5 shows the effects of iron and melanin on the amount of MDA of the co-culture. Incubation of the co-culture with the iron-melanin complex resulted in a significant increase in the amount of MDA compared with the control. This increase was also prevented by the addition of deferoxamine mesylate. The Fe^{3+}-ADP complex alone had no effect on the amount of MDA.

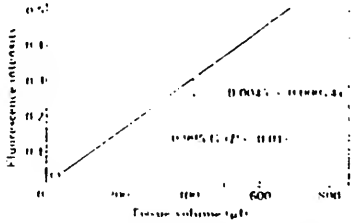

Figure 1. Correlation between fluorescence intensity and amount of dissociated cells used from the co-culture for the determination of lipid peroxidation. A linear relationship was obtained.

Discussion

A more extensive growth of dopaminergic neuronal fibers was observed in our nigrostriatal co-culture from the rat embryonic brain compared with conventional cultures of ventral mesencephalon. Therefore, the nigrostriatal co-culture system used here seems to be simulating more closely the in vivo status of the nigrostriatal system, and is more suitable for examining the toxic effects of candidate substances for PD on dopaminergic neurons. Furthermore, in nigrostriatal co-cultures, striatal cells stimulate the growth of the dopaminergic neurons (Prochiantz *et al*., 1979). Our present results indicate that iron alone is neurotoxic to cultured neurons in a dose-dependent manner. However, the toxic effect of iron is not restricted to dopaminergic neurons, since incubation with a higher concentration (> 50 μM) was also toxic for the TH-negative cells. A high concentration of free iron seems potentially harmful to all types of cells, but a lower concentration of iron may induce preferential damage of the dopaminergic neurons by an interaction with dopamine or neuromelanin. The loss of TH positive cells may in part be mediated by metabolic changes in the background cells. However, we think this possibility is not very likely because at concentrations of up to 25 μM of iron, the density of background cells appeared normal under phase contrast microscopy. If loss of TH positive cells is mainly mediated by the background cells, they should show more extensive damage than the TH-positive cells; this was not the case. Addition of dopamine melanin to the iron-ADP complex resulted in more extensive damage to TH positive cells (see Figure 4). Furthermore, an increase in the amount of MDA (more precisely thiobarbituric acid reactive product) was observed only when dopamine melanin co-existed with the Fe^{3+}-ADP complex (see Figure 5). Thus, iron in small amounts may induce neuronal damage by mechanisms other than lipid peroxidation. The increase in iron in PD reported in the literature is of interest since hydroxyl radical formation could be enhanced in diseased brain regions due to an interaction between ferrous iron and hydrogen peroxide formed by the action of MAO, as suggested by Ruderer & Youdim (1986). However, our study suggests that an

The CHAIRMAN. The committee stands in recess.
[Whereupon, at 11:07 a.m., the committee was adjourned.]

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